

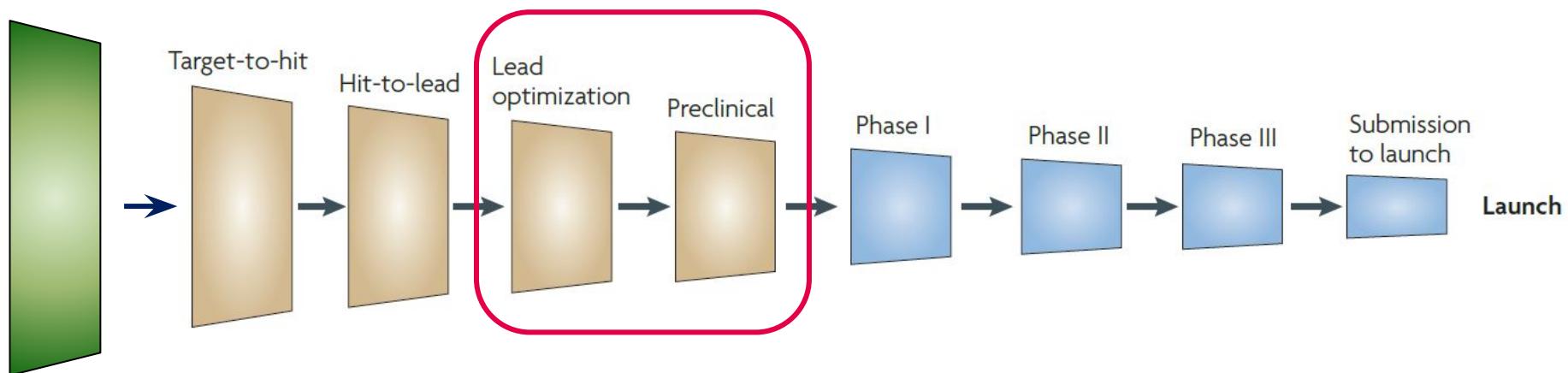
What efficacy and safety profiles can we expect

*Mathematical and Computational Biology in Drug Discovery
(MCBDD) Module IV*

*Dr. Jitao David Zhang
May 2024*

Where are we now

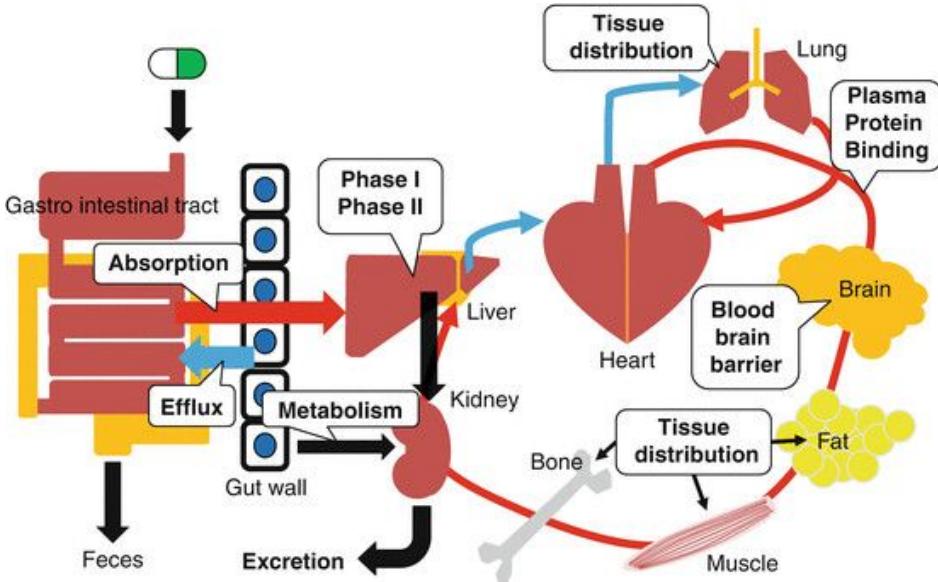
Target identification & assessment



Goal: we want to select **one compound** from a few (~ 10^2 - 10^0) for entry in human.

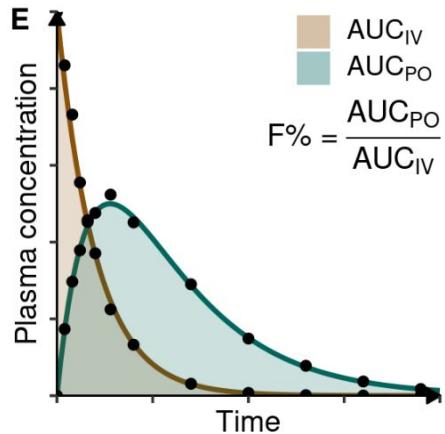
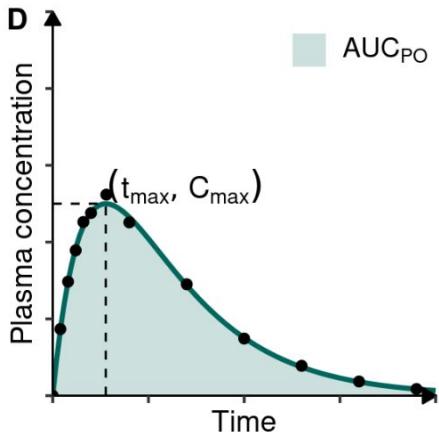
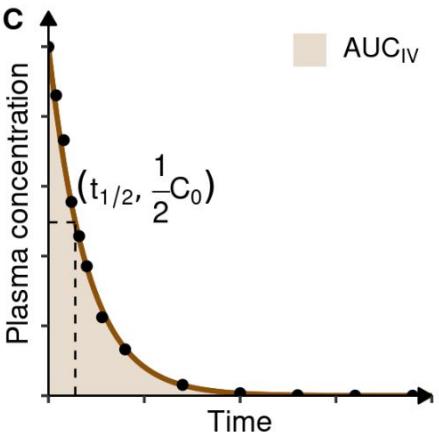
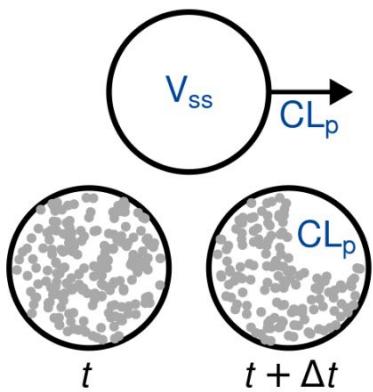
Key factors to consider in selecting compounds

- Efficacy and pharmacodynamics(PD)
- Pharmacokinetics (PK)
 - Absorption
 - Distribution
 - Metabolism
 - Excretion
- Toxicology



Key PK parameters: V_{ss}, CL_p, t_{1/2}, t_{max}, C_{max}, and F

B



V_{ss}	Volume of distribution at steady state.	C_{max}	Maximum plasma concentration.
CL_p	Plasma clearance.	t_{max}	Time point in which the Cmax is measured.
t_{1/2}	Half-life, time for a substance to reach the half concentration of the initial value (C ₀).	F% (or F)	Bioavailability, the percentage of the administered compound reaching systemic circulation.

The Hill function as a typical PD model

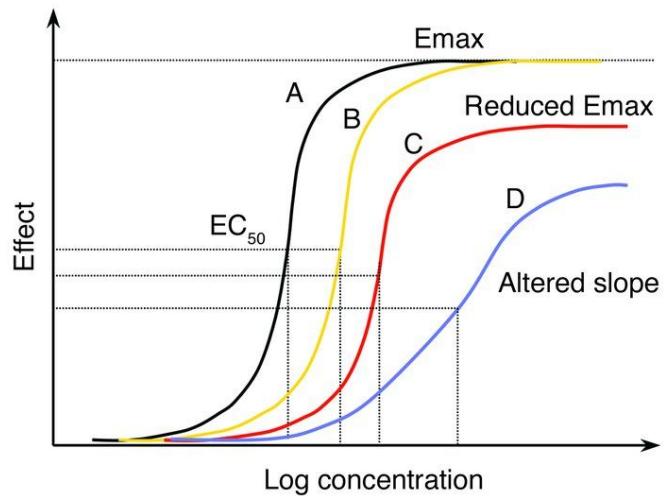
- The Hill function is one of the mostly useful non-linear functions to model biological systems.
- In its general form, H_{max} indicates the maximal value to which the function is asymptotic, n is the shape parameter (known as the Hill's coefficient), and k is the reflection point, often abbreviated as XC_{50} ($X=I, E, C, \dots$), the half-saturation constant.
- The Michaelis-Menten model is a special case of the Hill function with $n=1$.

$$H = H_{max} \frac{x^n}{k^n + x^n}$$

General form of the Hill function

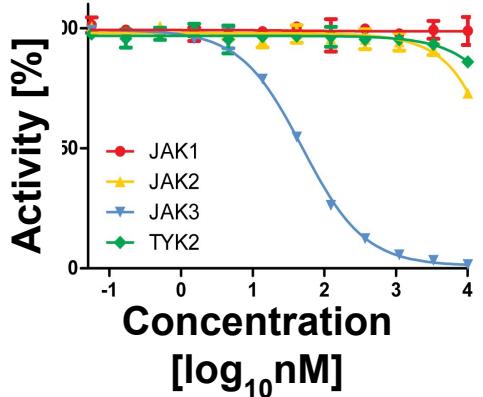
$$\begin{aligned} E &= E_{max} \frac{[L]^n}{EC_{50}^n + [L]^n} \\ &= E_{max} \frac{1}{1 + \left(\frac{EC_{50}}{[L]}\right)^n} \end{aligned}$$

Modelling dose-dependent effect

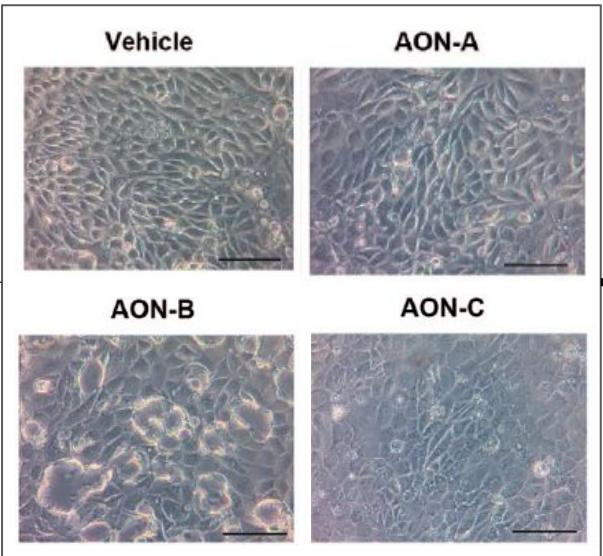


White. J Clin Invest. 2004;113(8):1084-1092.
<https://doi.org/10.1172/JCI21682>.

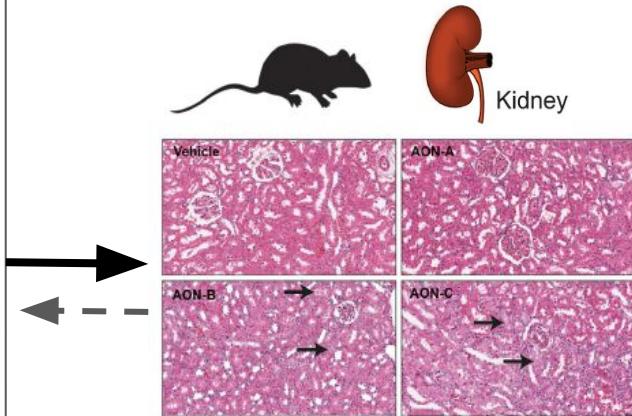
Classical workflow of efficacy and toxicity assessment



Biochemical &
biophysical assays



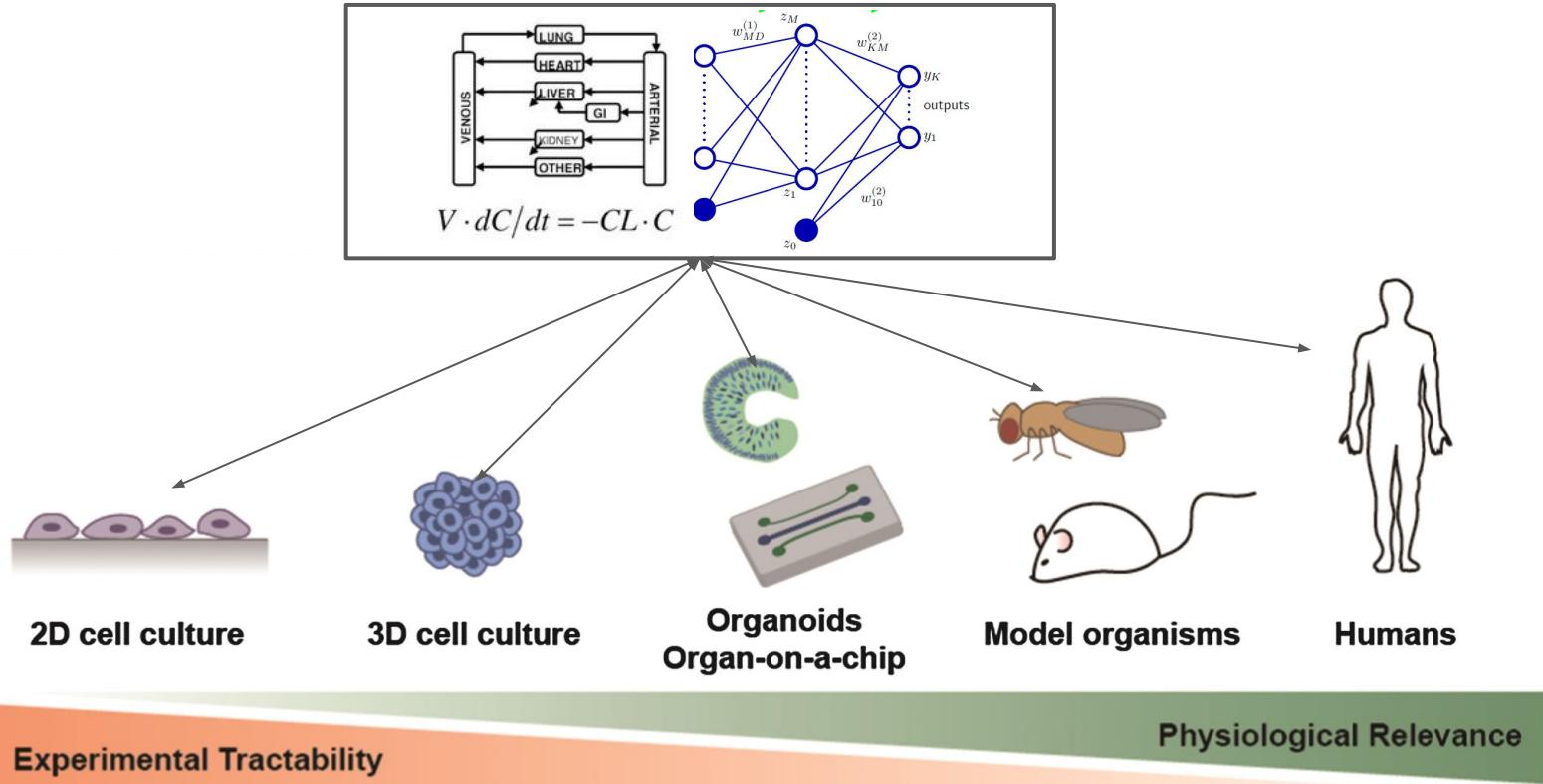
Cellular assays
(*in vitro*)



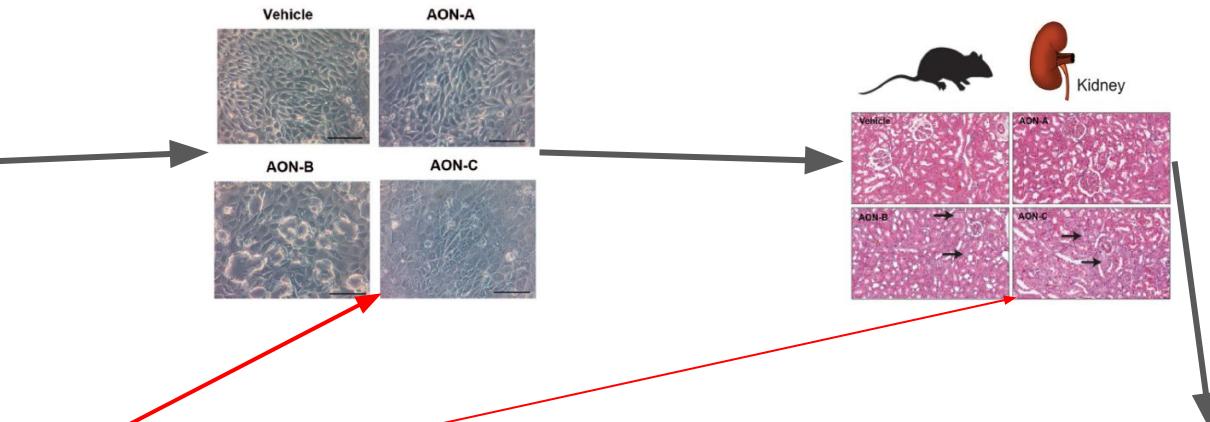
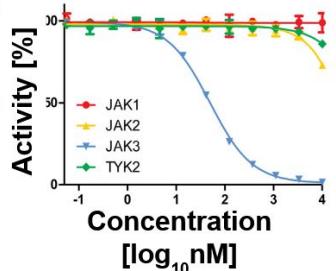
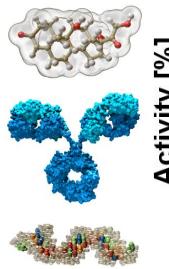
Animal
experiments
(*in vivo*)

→ Usual workflow
 ← - - Assay development

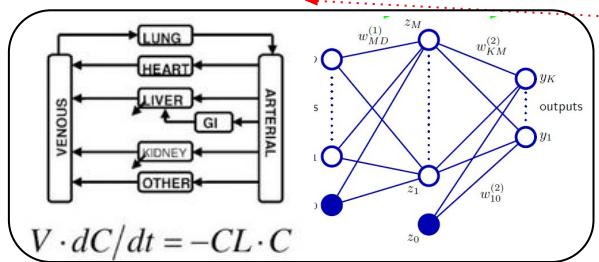
Biological and computational models of human diseases



Computational methods empower efficacy and toxicity assessment



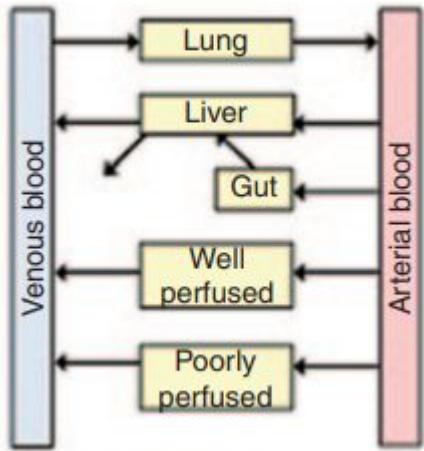
High-throughput
technologies (omics,
microscopy, etc.)



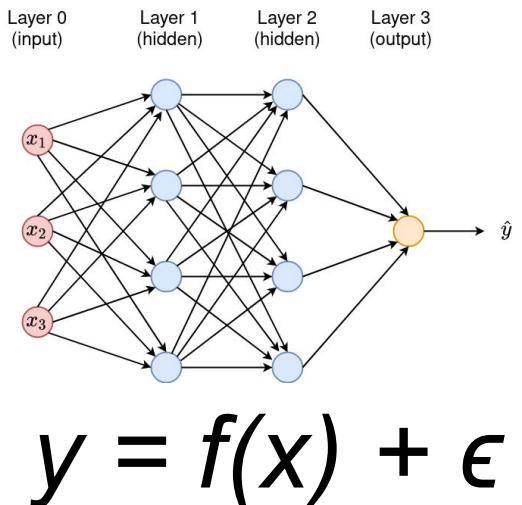
Mechanistic, causal,
and statistical models



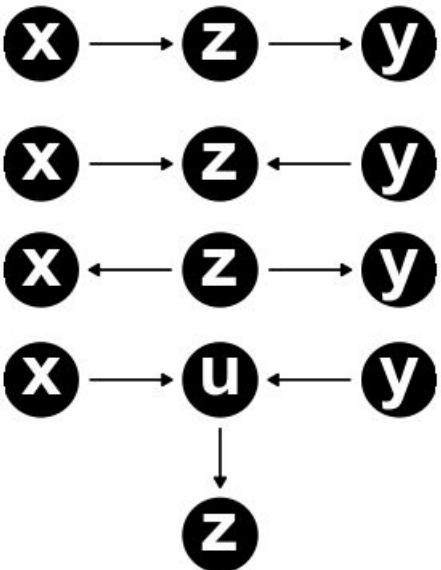
Three types of computational models



Mechanistic models

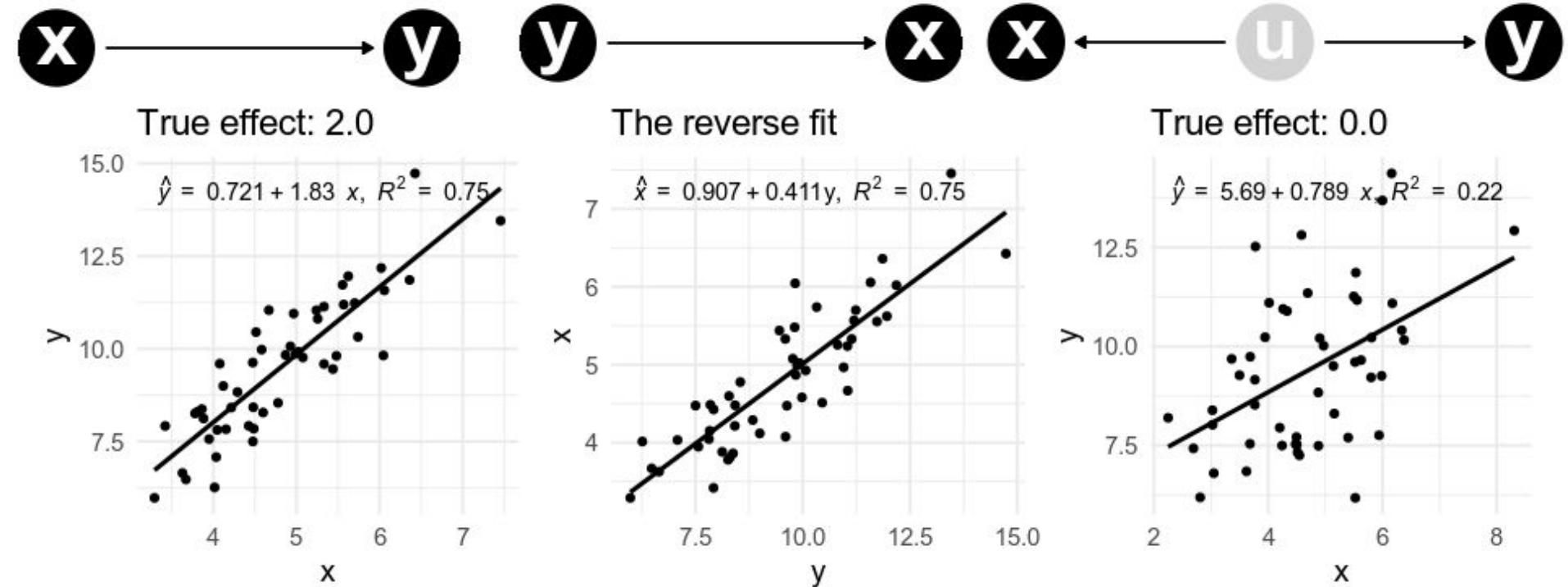


Statistical and
machine-learning models



Causal models

Correlation is caused by causation, confounding, coincidence, or conspiracy



Statistical models alone cannot derive causality from correlation

We learn causality by (1) listing models explicitly and (2) manipulating a variable and observe the outcomes

Model 1



Model 2

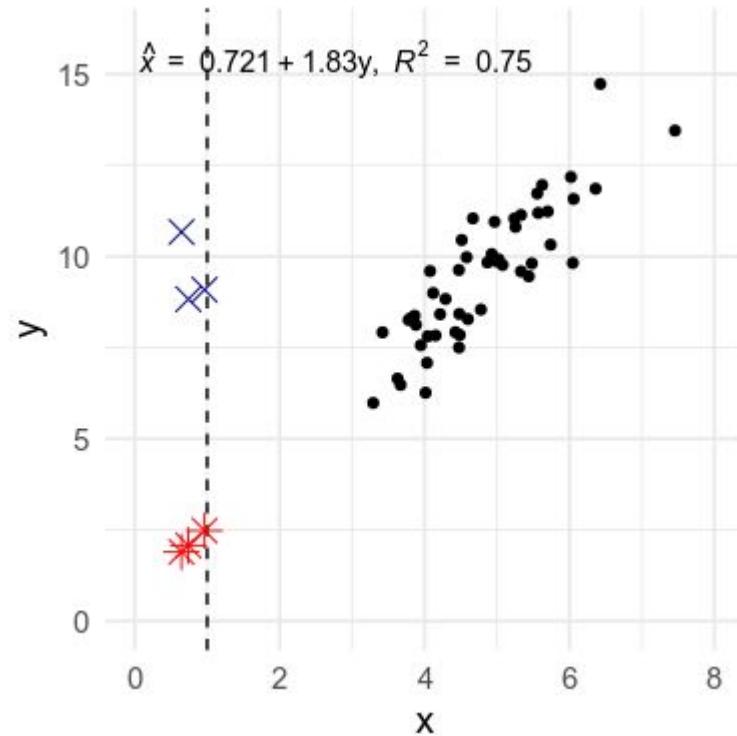


Model 3

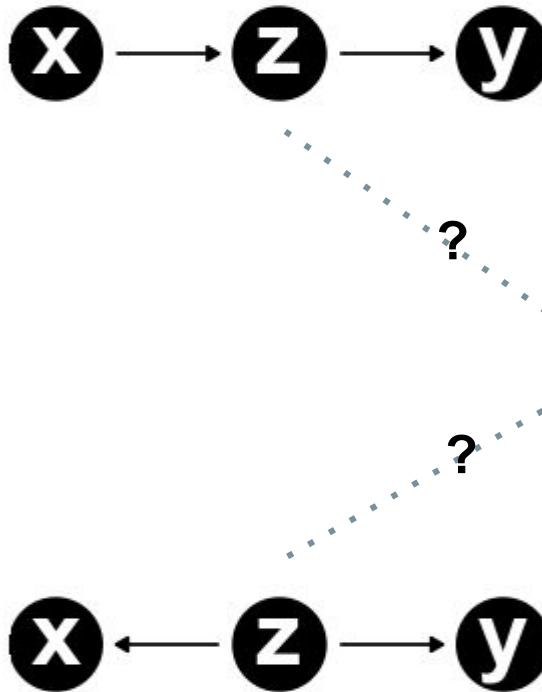


Assume that the data is generated by either Model 1, or Model 2, or Model 3. And assume that we can manipulate the value of X by setting it to 1.0 (the dash line).

Question: which outcomes (red stars or blue crosses) would support which models? Why?

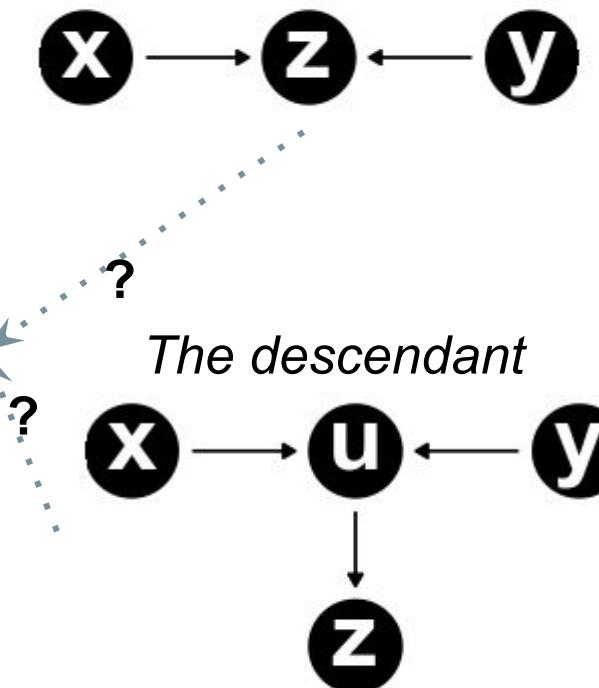


Causality is crucial for drug discovery



Biomarker, tox study, pathology,
omics data, real-world data, ...

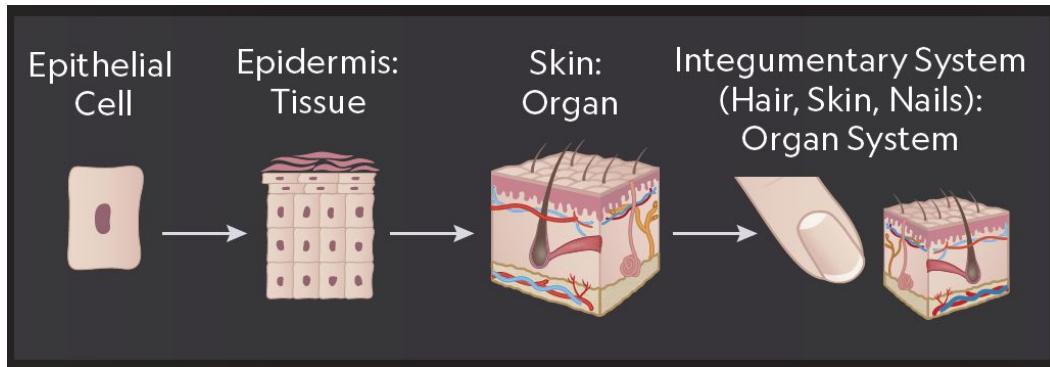
	x	z	y
1	0.835386320	1	-0.73897252
2	-0.005354014	-1	-0.82972315
3	0.058788286	1	0.76213369
4	-1.015602246	-1	-0.05951719
5	-0.339569780	-1	-0.11745910
6	-0.041077979	-1	-1.28243716
7	0.363740407	1	-0.30570762
8	0.119496314	-1	-1.19932461
9	0.257108454	-1	-1.06044066
10	0.304537158	-1	-0.43396492



We need both models (knowledge + assumptions) and data to infer causality.

End of Lecture 9 on 3.5.2024

Complexity Increases Through a System

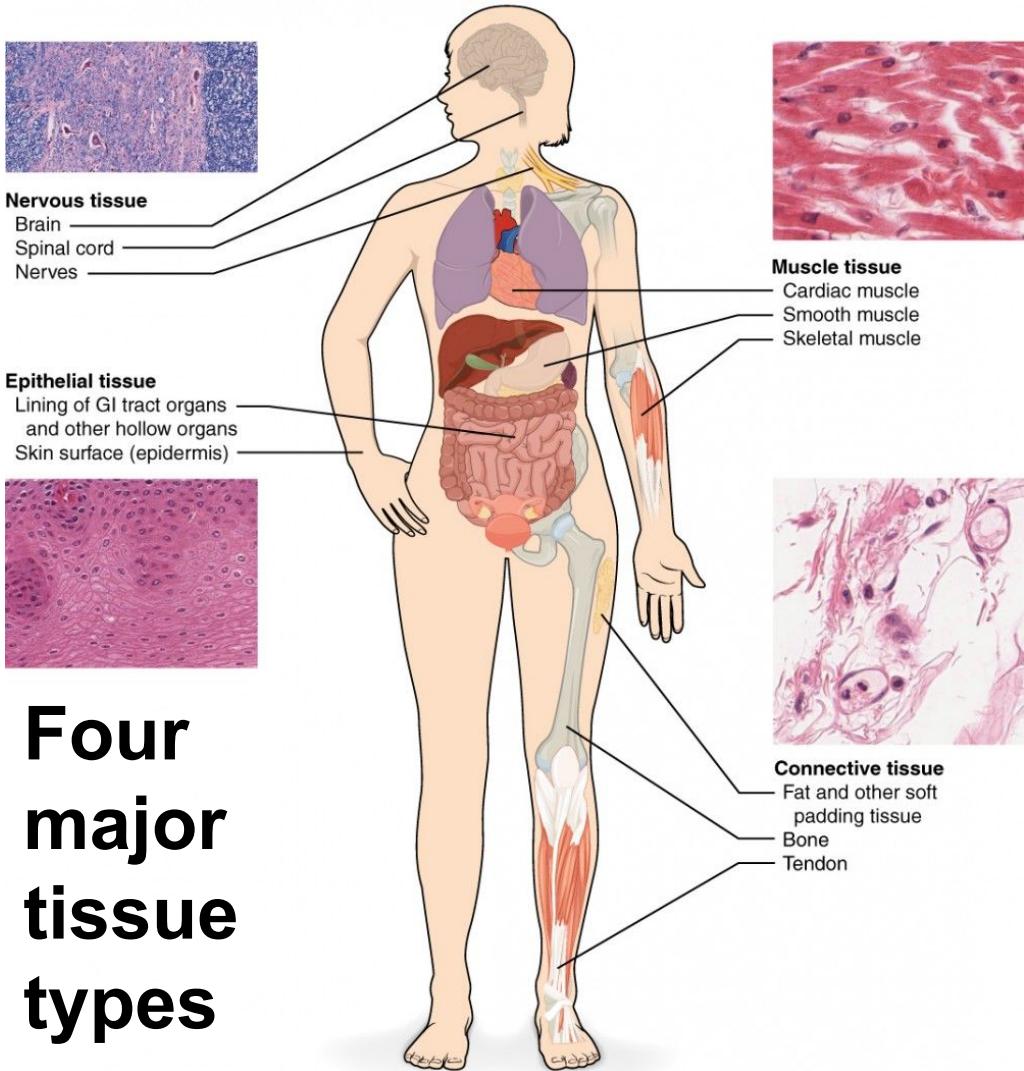
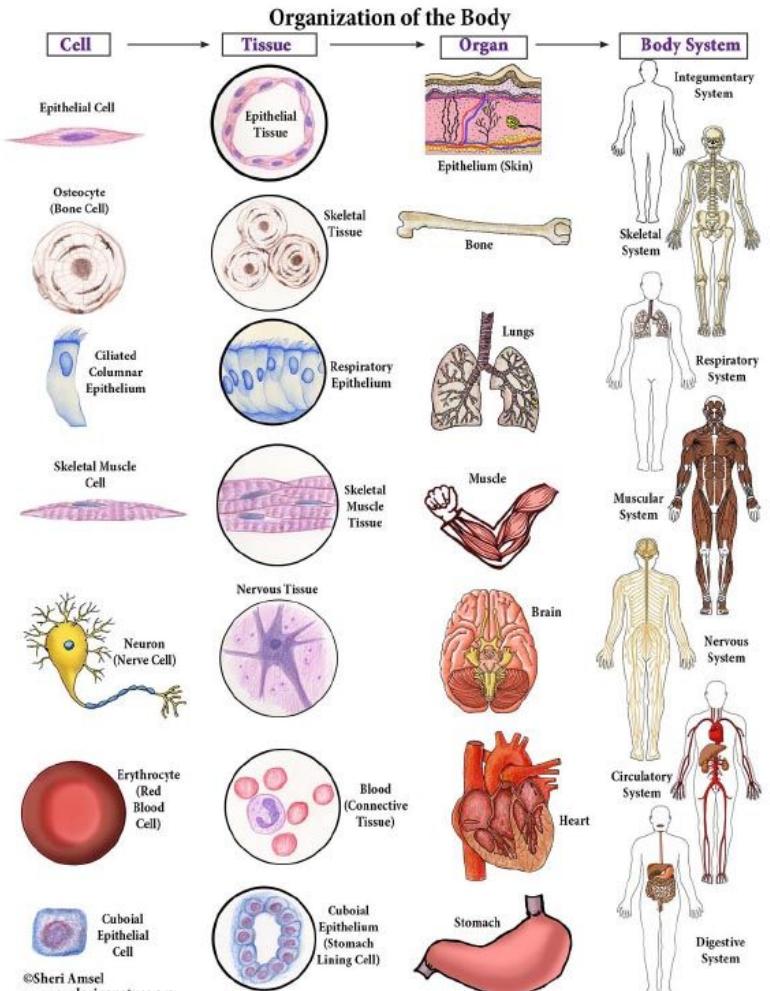


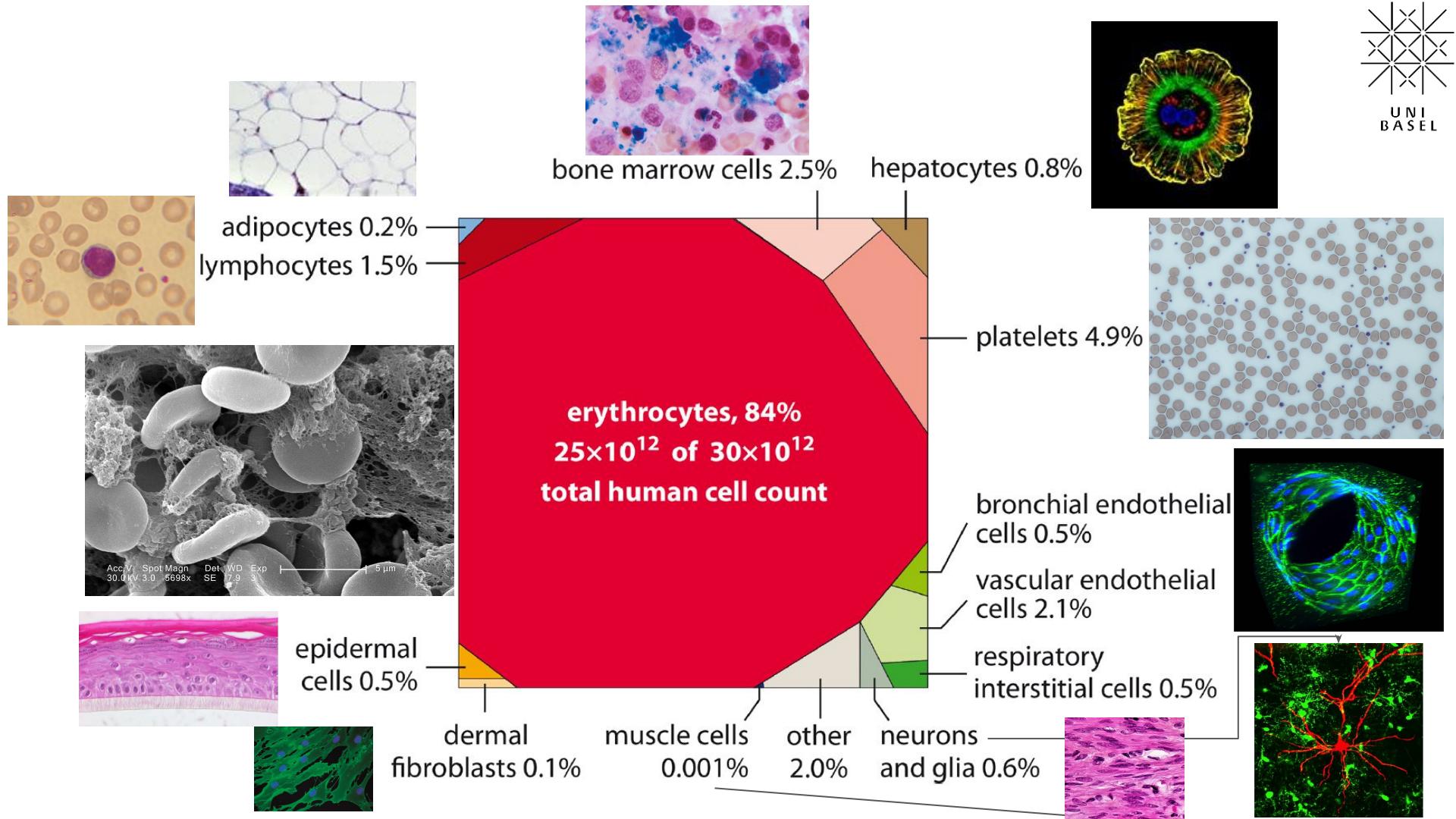
Cells: basic building blocks, variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues





What's in a drop of blood? Ask a doctor or a biologist!

Plasma:

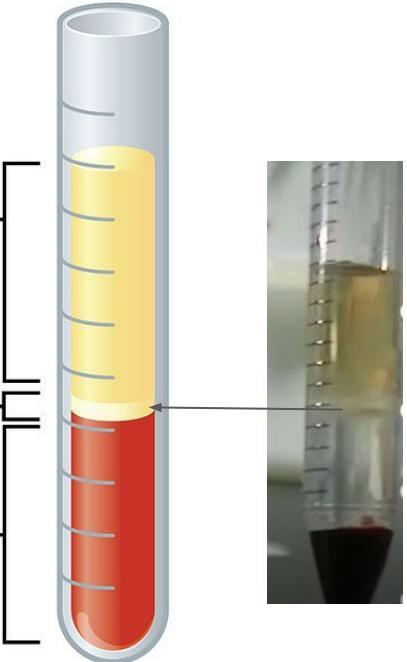
- Water, proteins, nutrients, hormones, etc.
- ~55%

Buffy coat:

- White blood cells, platelets
- <1%

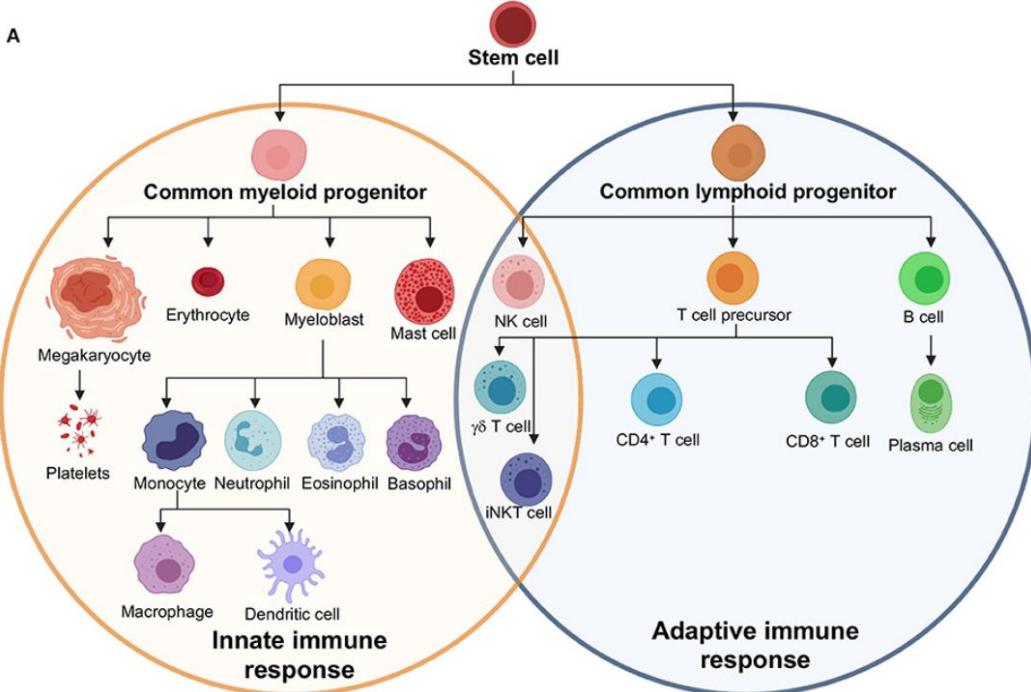
Hematocrit:

- Red blood cells



Normal Blood:

♀ 37%–47% hematocrit
 ♂ 42%–52% hematocrit

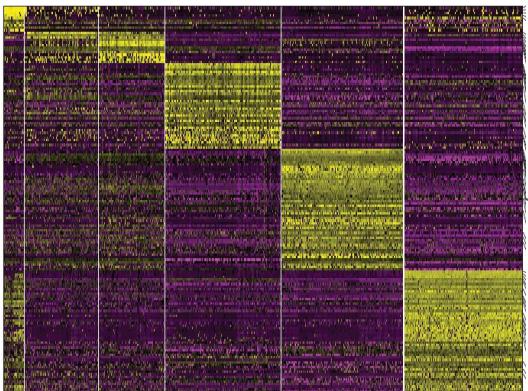


What's in a drop of blood? Count the genes!



Sequencing

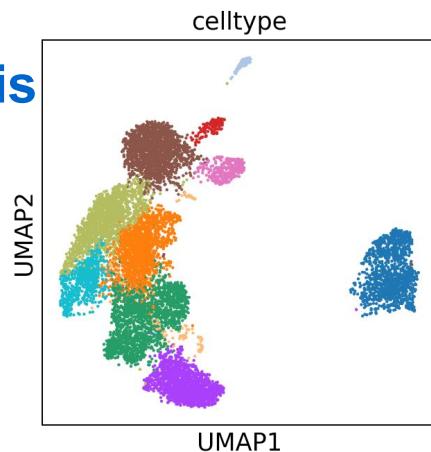
Genes



Cells

Low Expression  High Expression

Data analysis

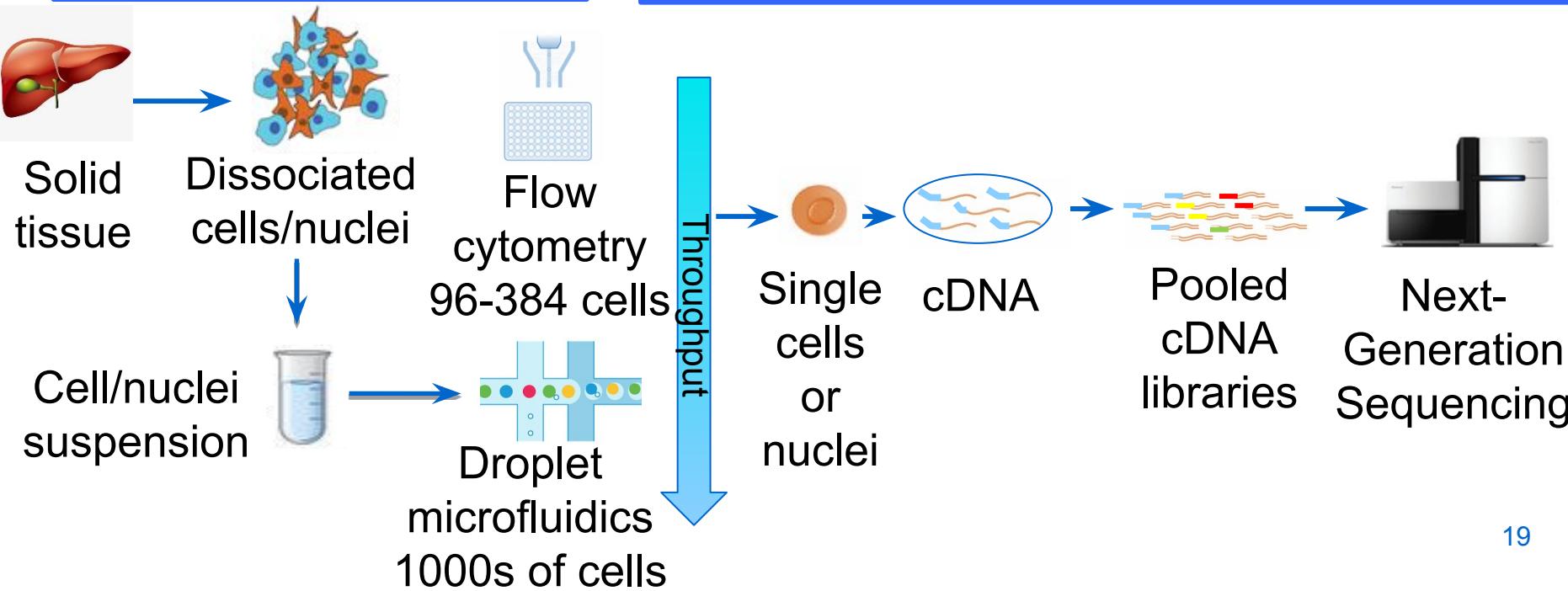


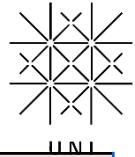
- B-cell
- CD4 T-cell
- CD8 T-cell
- DC
- NK cell
- monocyte CD14+
- monocyte CD16+
- naive CD4 T-cell
- naive CD8 T-cell
- pDC
- unknown

Single-cell sequencing (scSeq) workflow

Tissue dissociation

Single cell capture and transcriptome sequencing





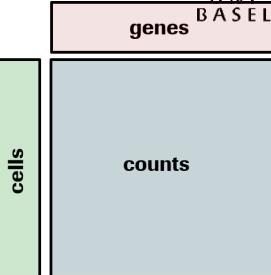
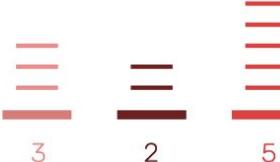
A linearized workflow of scSeq data analysis

From short reads to gene-cell matrix

Alignment

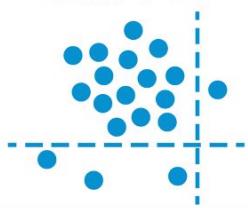


Quantification

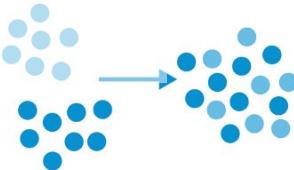


QC, filtering & normalization,
dimensionality reduction, and
clustering

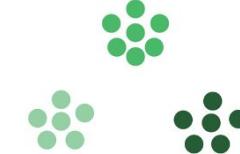
Quality control



Normalisation



Clustering



Downstream analysis

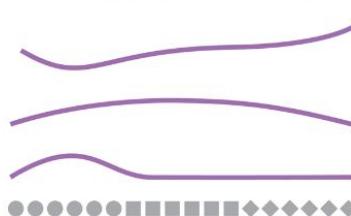
Differential expression



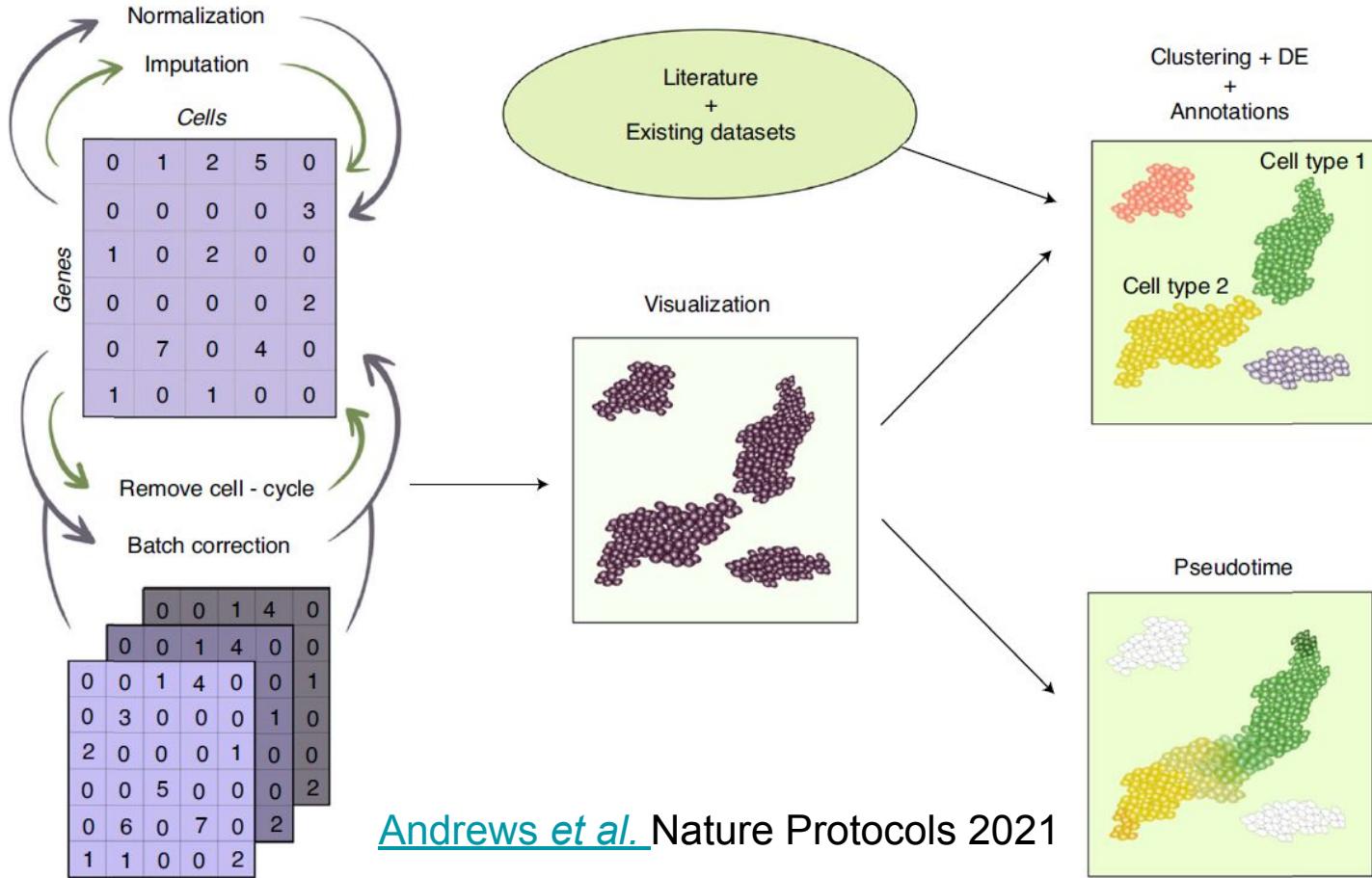
Marker genes



Expression patterns

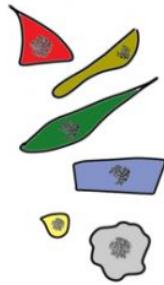
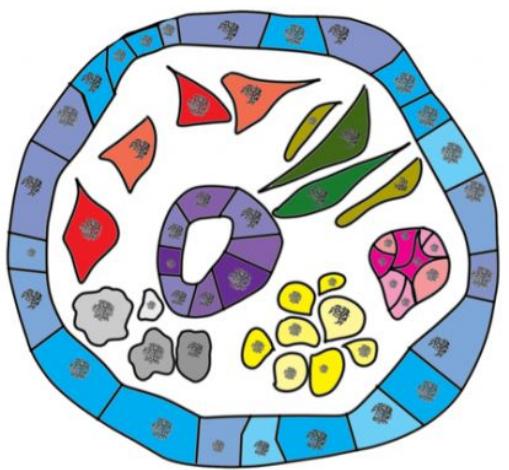


Overview of the computational workflow

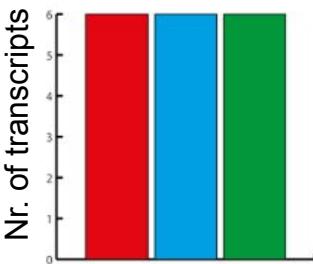


[Andrews et al.](#) Nature Protocols 2021

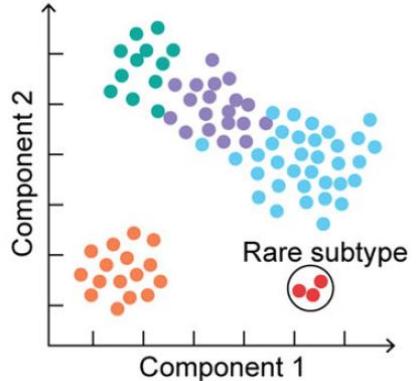
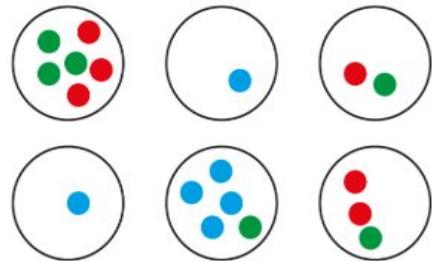
Single-cell biology benefits both disease understanding and drug discovery



Bulk analysis

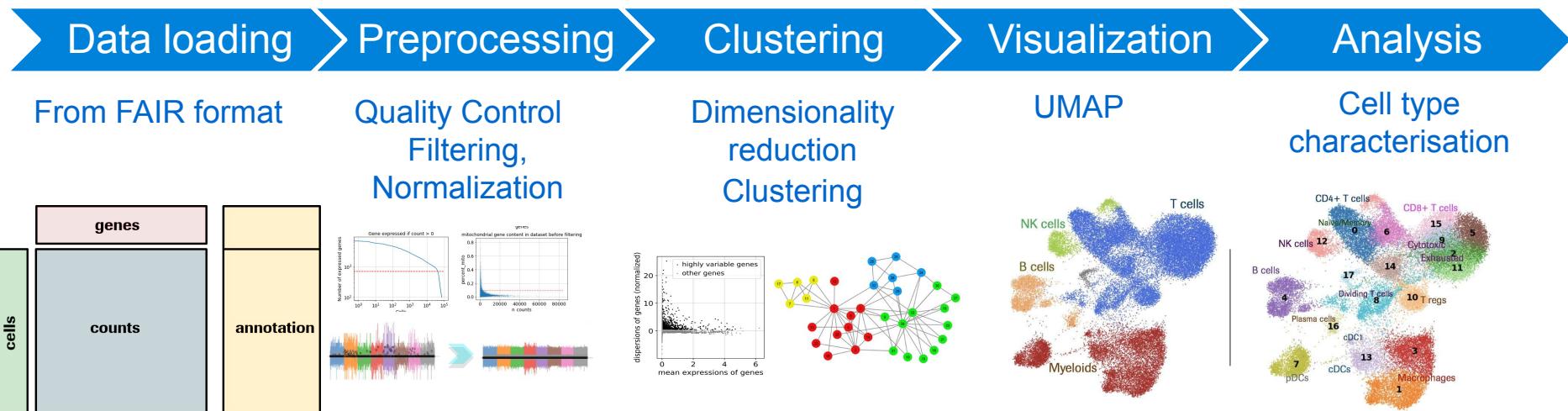


Single cell transcriptome analysis

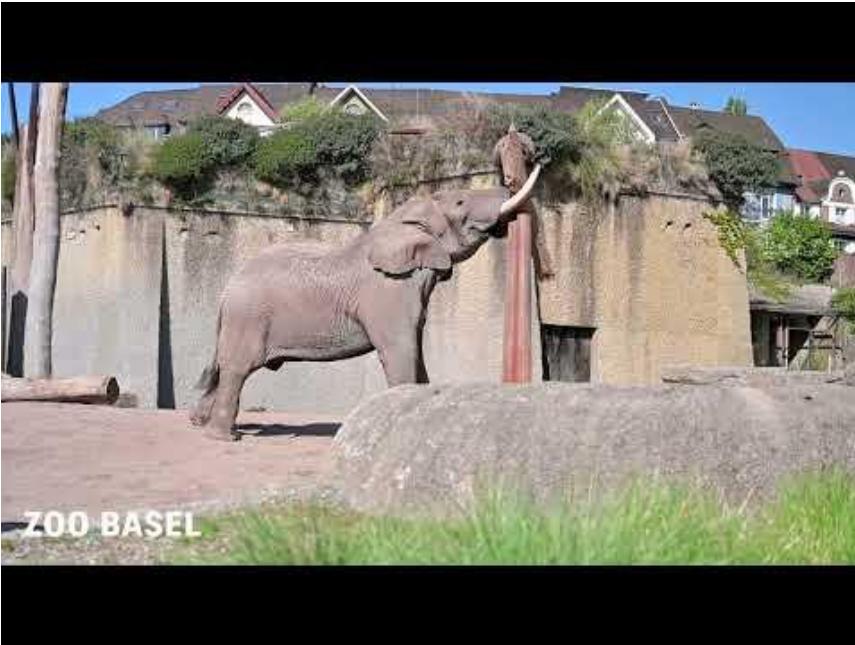


BESCA: An open-source Python package for single-cell gene expression analysis

An automated standard workflow

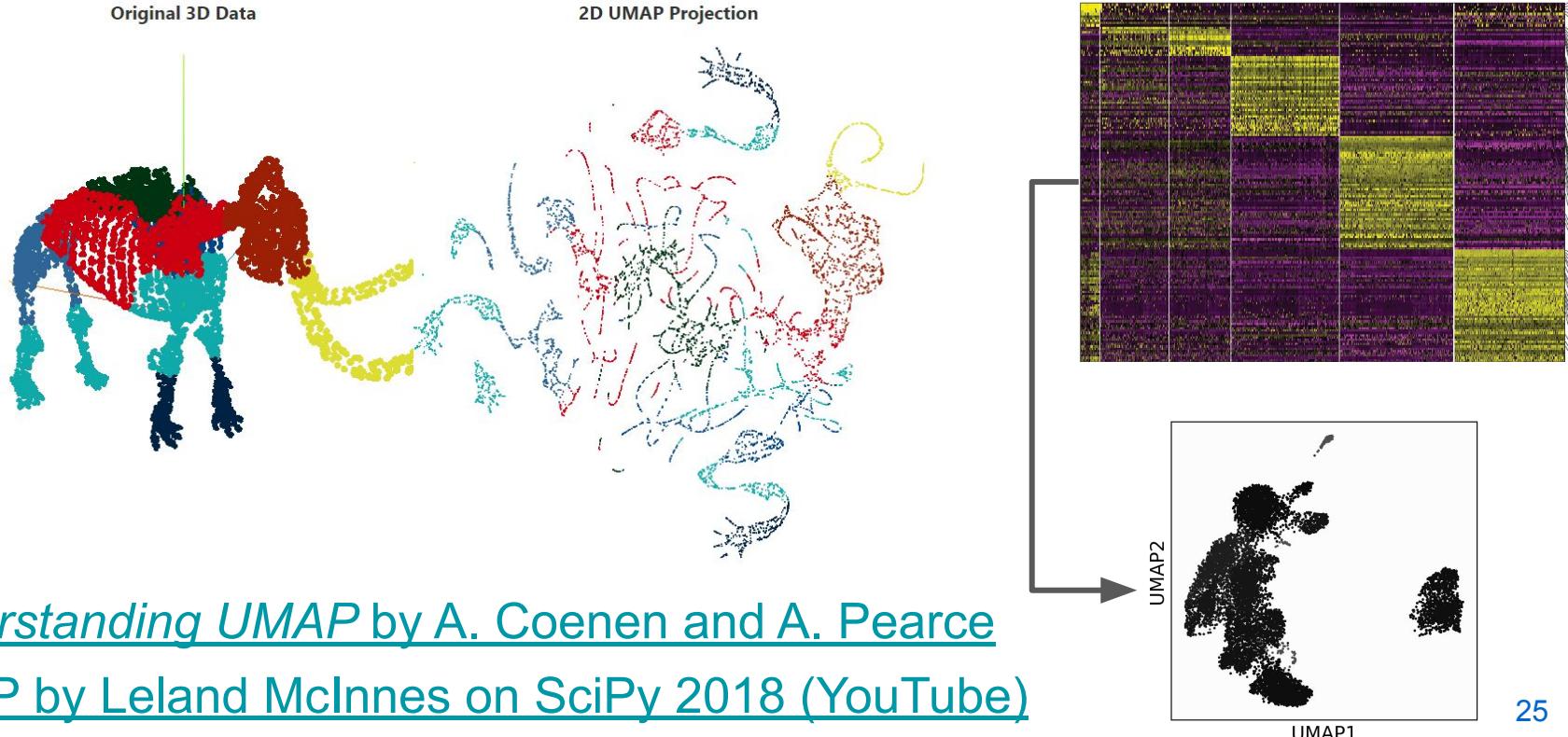


How to represent voxels with pixels?



The elephant bull *Tusker* (1992-2023) at Zolli Basel plays with a tree trunk on a post (2022)

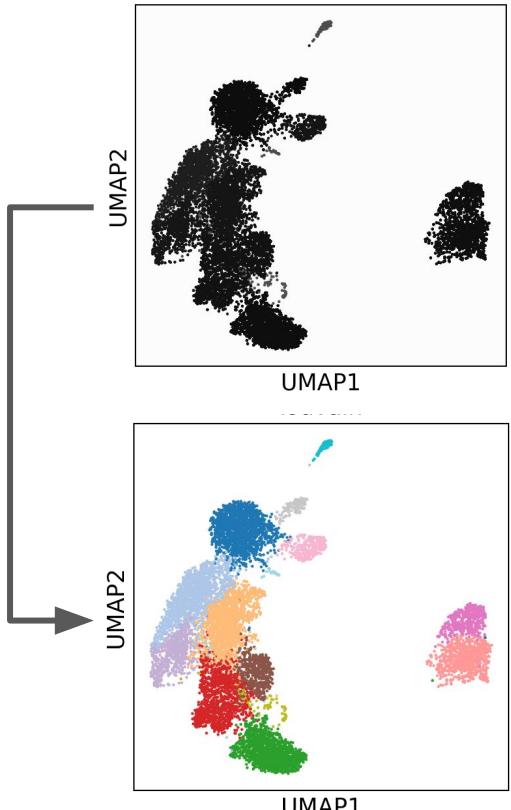
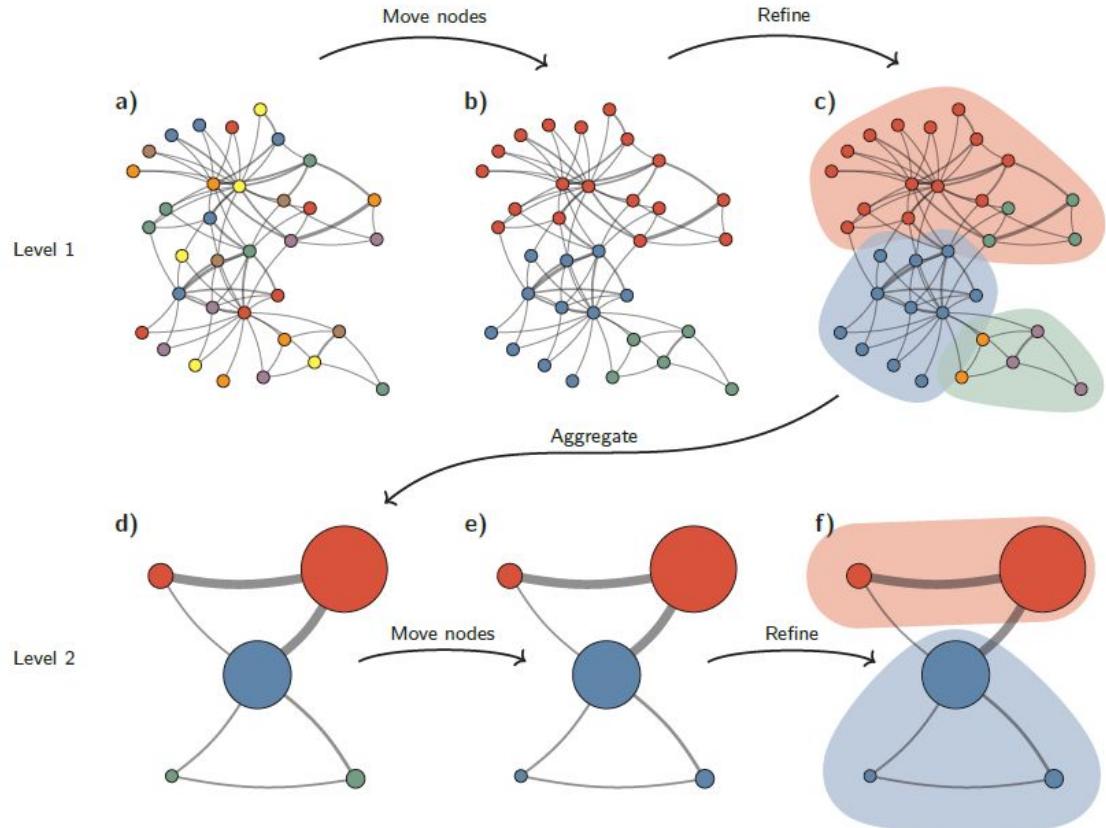
Uniform Manifold Approximation and Projection (UMAP) for dimension reduction



[Understanding UMAP](#) by A. Coenen and A. Pearce

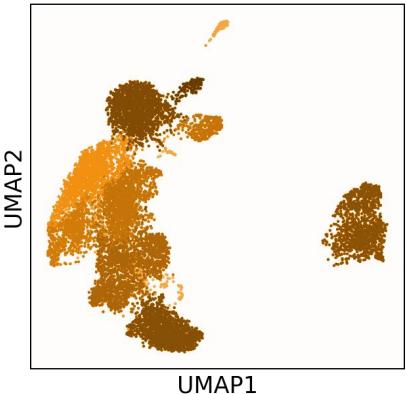
[UMAP](#) by Leland McInnes on SciPy 2018 (YouTube)

The Leiden Algorithm for Community Detection

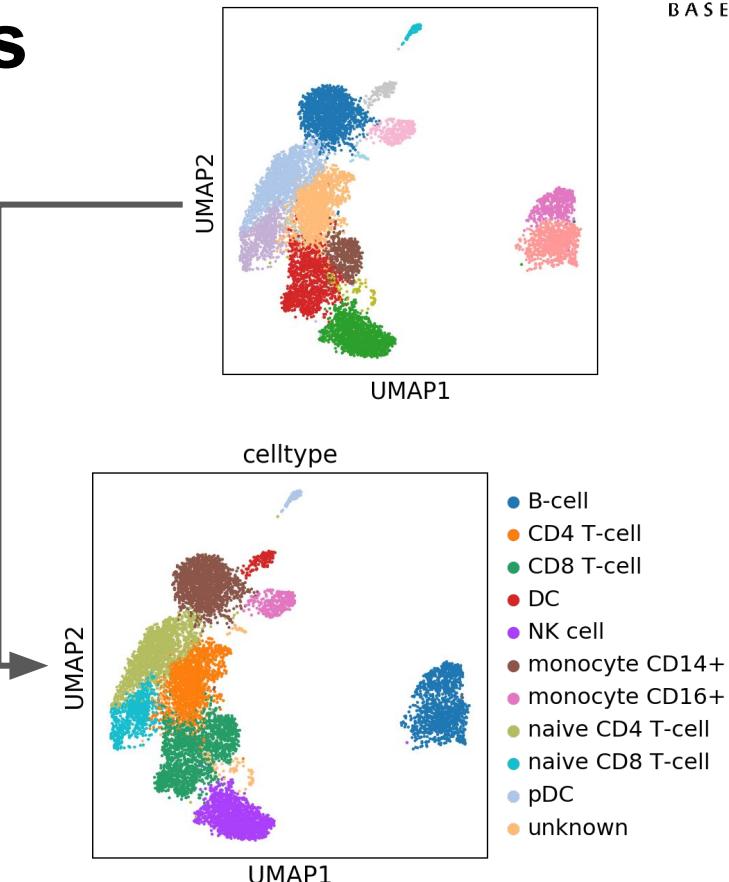


Biological knowledge and visual inspection is used to annotate cell types

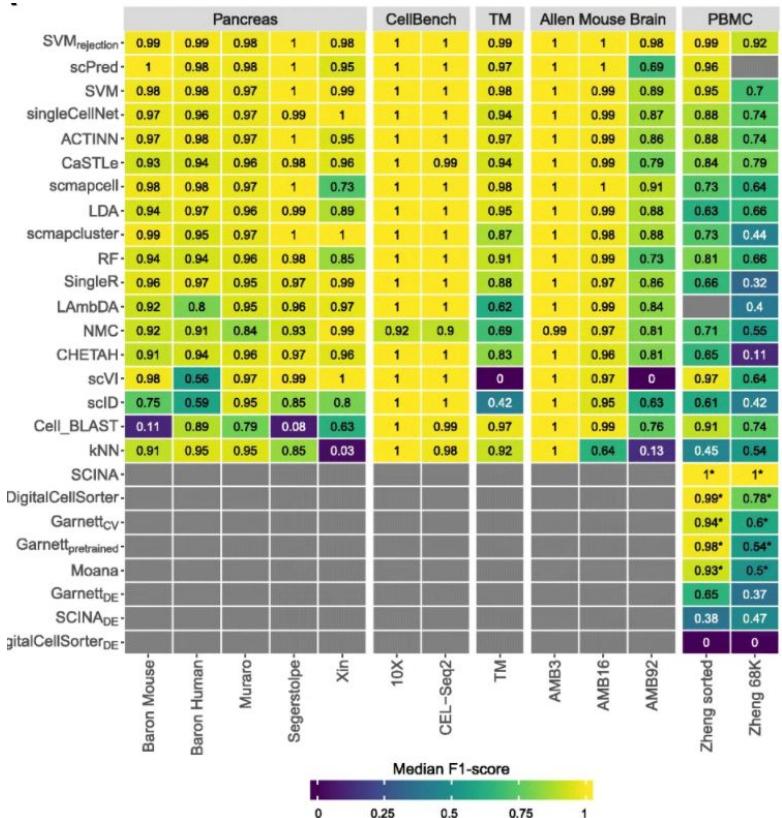
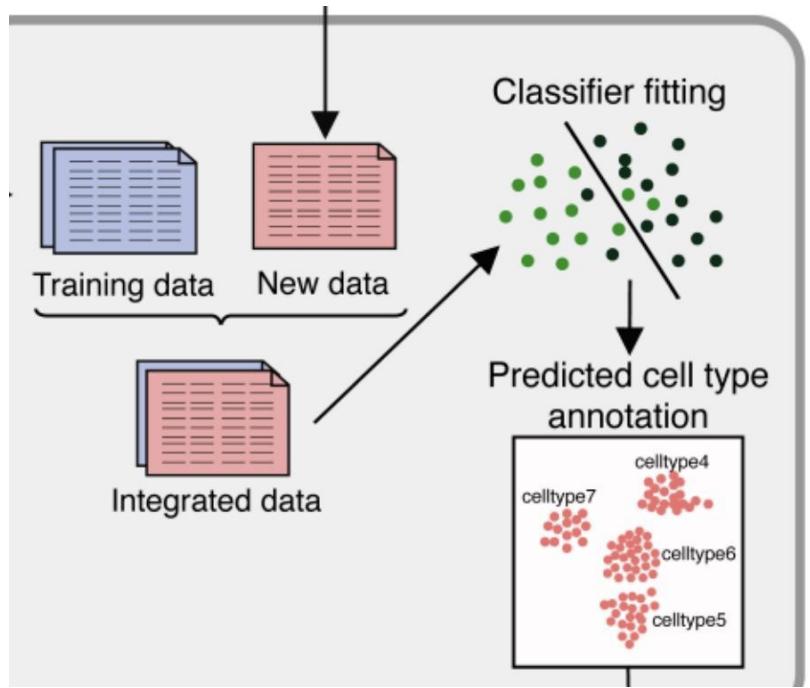
Heatmap
of gene X



lymphocyte	PTPRC							
myeloid	S100A8	S100A9	CST3					
Bcell	CD19	CD79A	MS4A1					
Tcells	CD3E	CD3G	CD3D					
CD4	CD4							
CD8	CD8A	CD8B						
NKcell	NKG7	GNLY	NCAM1					
monocyte	CST3	CSF1R	ITGAM	CD14	FCGR3A	FCGR3B		
macrophage	CD14	IL1B	LYZ	CD163	ITGAX	CD68	CSF1R	FCGR3A

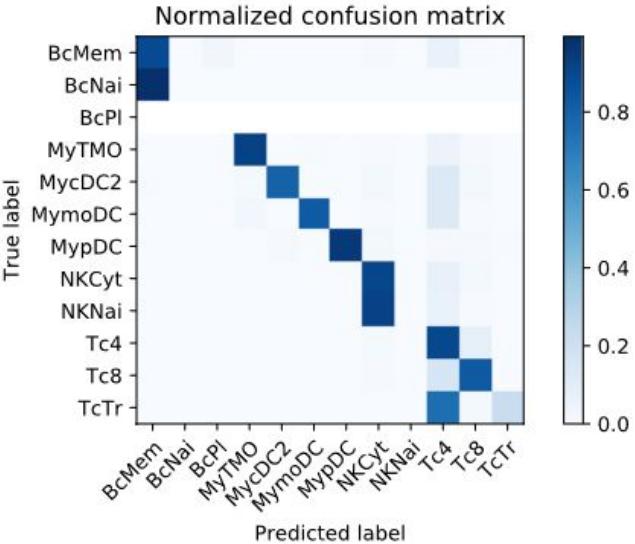
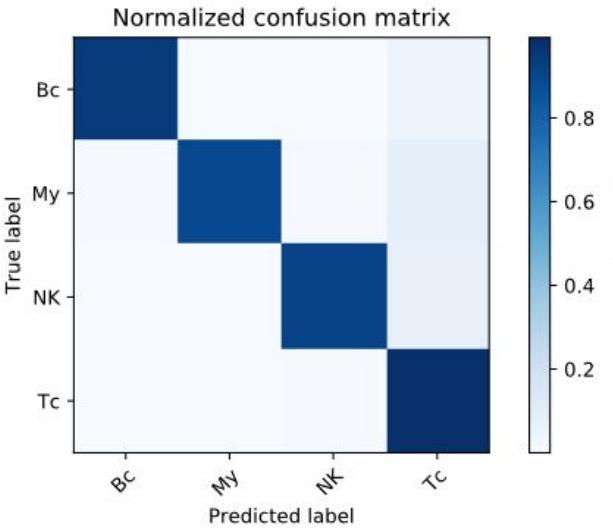
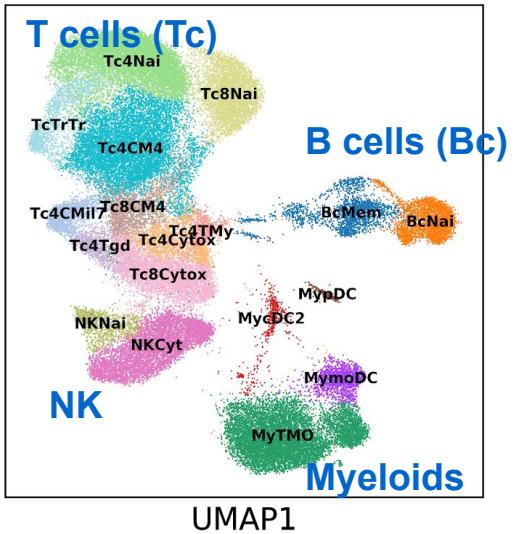


Cell type annotation with machine learning



A PBMC example of cell type annotation

UMAP2



- Broad level cell types, including B cells (Bc), Myeloid (My), NK cells (NK) and T cells (Tc), are successfully predicted.
- Missing and highly similar cell types cause challenges with increased granularity. Essential: reference data quality and knowledge of cell types. ²⁹

Single-cell biology is important in drug discovery

Disease understanding:

disease-specific cell types
and states



Target identification:

expression pattern in
health and disease across
cell types



Biomarker and patient stratification:

which genes should we measure
in which cell type(s)?



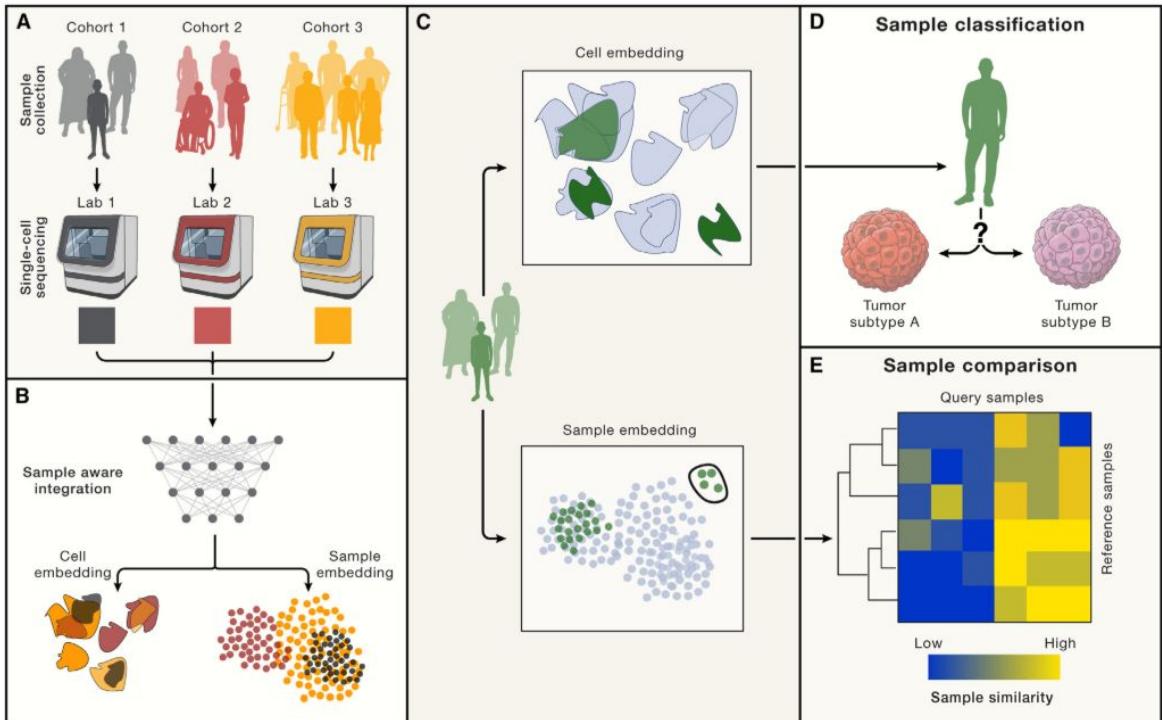
MoA and safety

modelling: perturbation effect at single-cell level

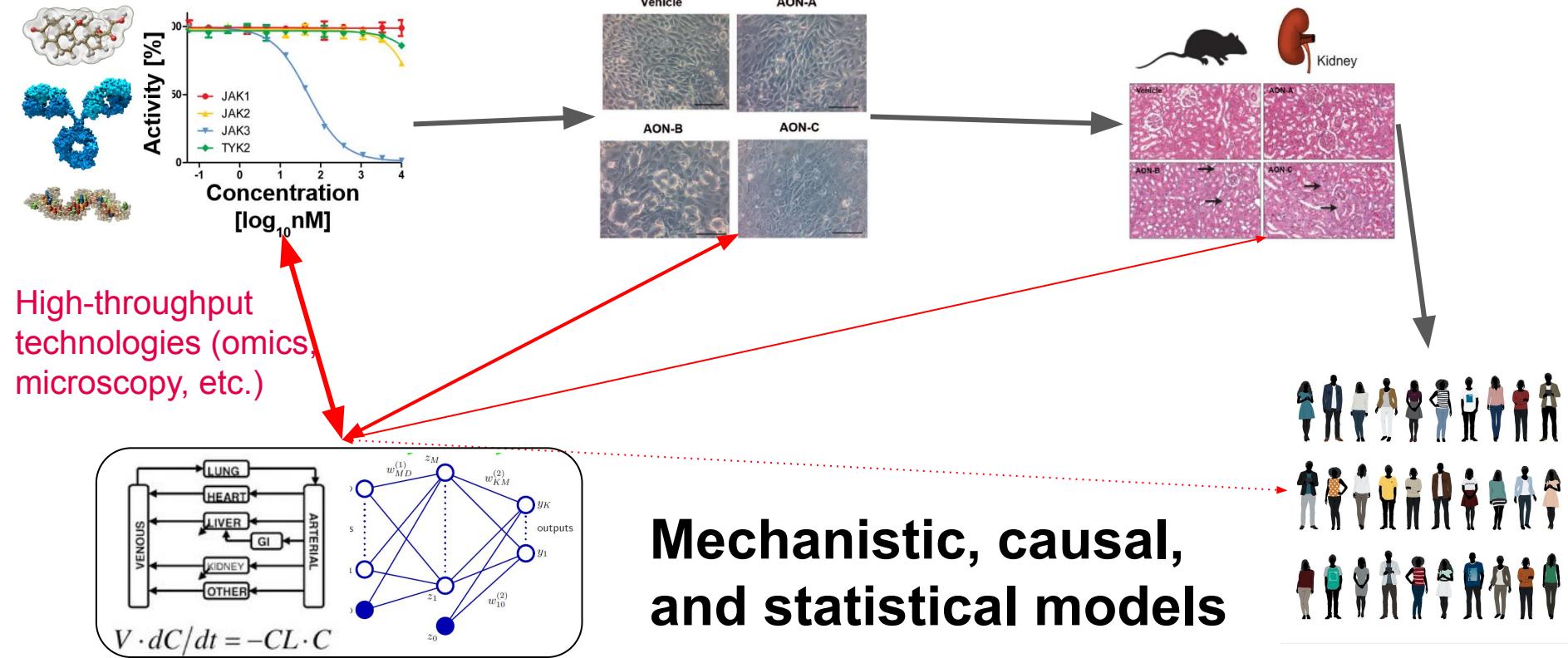


Reference mapping at population scale

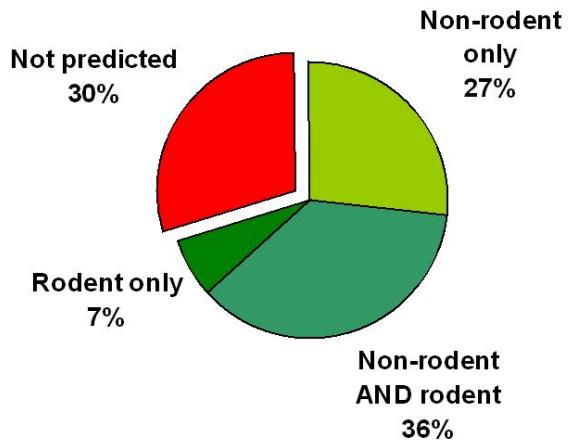
- The data platform Chan Zuckerberg CELL by GENE Discover ([CZ](#) [CELLxGENE](#)) provides data of 85 million cells as of April 2024 to be explored online.
- Much research and development now devotes to mapping data from different labs to reference datasets in order to annotate cells and samples in a (semi-)automated fashion



Computational methods empower efficacy and toxicity assessment



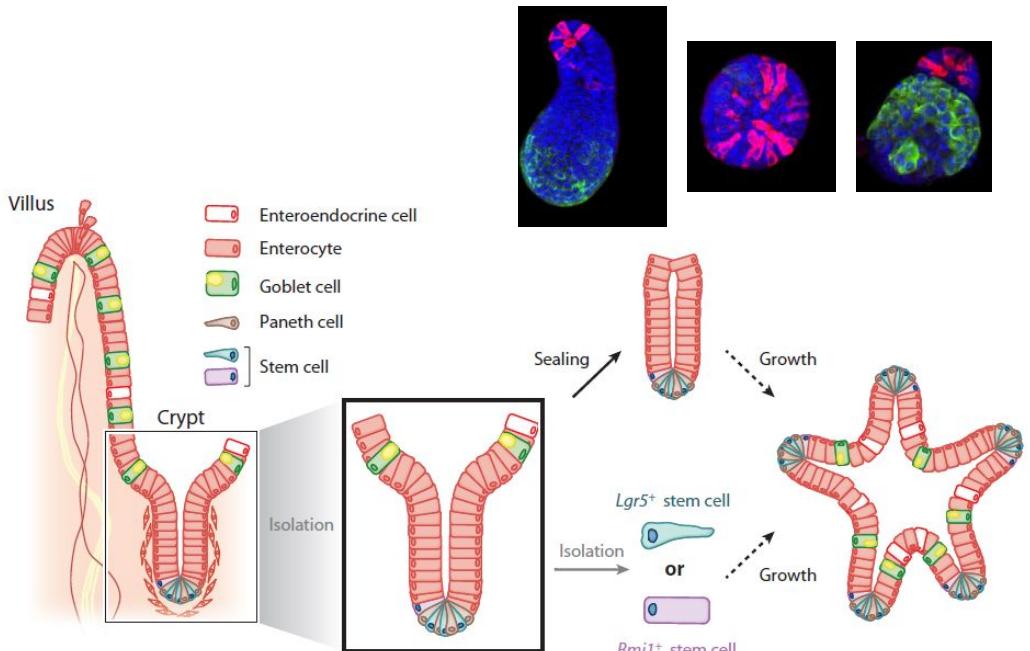
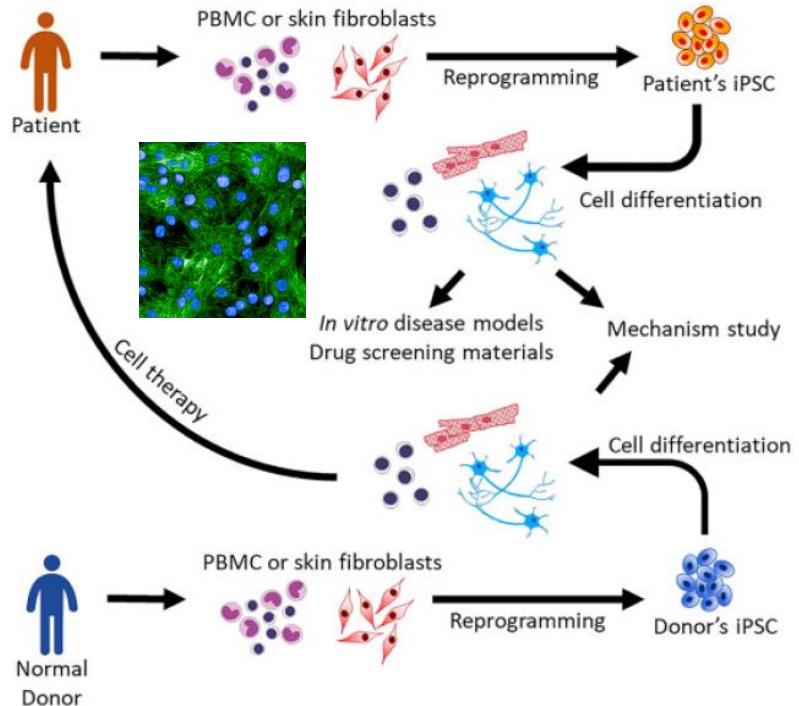
How predictive is animal safety testing for humans? It depends on modality and therapeutic classes.



[Regul Toxicol Pharmacol. 2000;32:56-67](https://doi.org/10.1016/j.reprotox.2009.08.001)

Target organ of ADRs	Small molecule drugs		Large molecule drugs	
	% of ADRs	% of correlation	% of ADRs	% of correlation
Gastrointestinal	21	80	14	19
Neurological	20	34	11	4
Hepatobiliary	11	73	8	21
Hematological	8	75	8	80
Cutaneous	5	56	9	22
Systemic	5	45	8	20
Cardiovascular	4	61	6	0
Ocular	5	64	5	83
Musculoskeletal	3	16	5	0
Metabolic	4	50	3	43
Faucal/oral	4	41	3	38
Urinary	3	61	3	14
Respiratory	1	45	5	32
Infection	0.4	100	6	68
Nasal	1	27	2	33
Application site reaction	1	100	3	81
Others	3	45	1	80

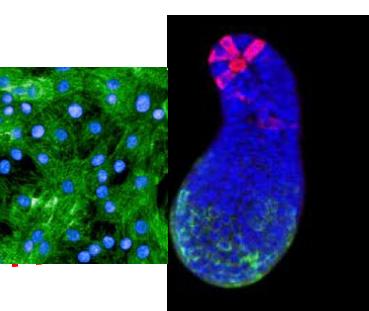
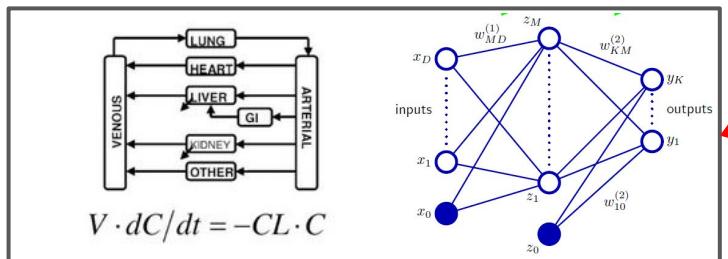
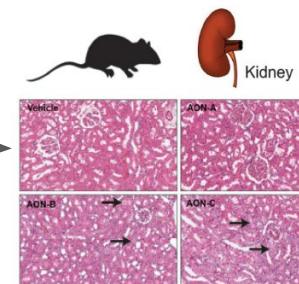
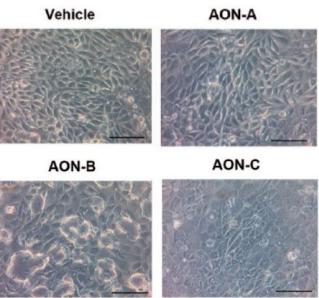
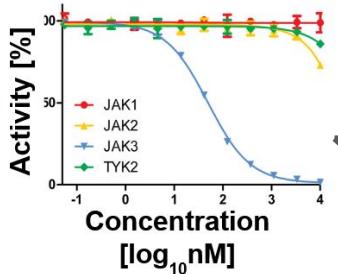
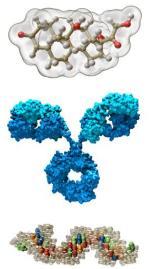
Stem cells and organoids empower efficacy and toxicity assessment



Small-intestinal organoids

Induced pluripotent stem-cells

Computational methods and novel biological models empower efficacy and toxicity assessment



Conclusions

- We predict efficacy and safety profiles of drugs by studying the mechanism and mode of action (MoA).
- The study of MoA involves building mechanistic, statistical, and causal models to predict what drug does to the body (pharmacodynamics) and what body does to the drug (pharmacokinetics).
- Bulk and single-cell RNA sequencing, and proteomics based on mass spectrometry (MS, in backup) are essential tools for understanding MoA.

Offline activities of Module IV (optional)

Perform your own single-cell data analysis to get first-hand experience working with high-dimensional biological data.

- If you are new to the topic, please use [the PBMC tutorial of Scanpy \(python\)](#) or [the PBMC tutorial of Seurat \(R\)](#).
- If you have experience with such data already, checkout [the NBIS workshop on single-cell sequencing data analysis](#) to cover advanced topics such as spatial transcriptomics and trajectory inference.

References

1. Figures: [Lumen Learning](#), [Exploring Nature](#), [National Geographic](#), [Platelet cells](#) (Graham Beards, CC-BY-SA 4.0), [Lymphocytes](#) (Nicolas Grandjean, CC-BY-SA 3.0), [Adipocytes](#) (Public Domain), [Hepatocytes](#) (CC-BY-NC 2.0), [Neurons and Glia](#) (Public Domain), [Blood](#) (CC 3.0), [Blood Cells](#) (By A. Rad and M. Häggström. CC-BY-SA 3.0 license), [A selective JAK3 inhibitor](#) (London Lab/Weizmann institute)
2. Sender, Ron, Shai Fuchs, and Ron Milo. 2016. "Revised Estimates for the Number of Human and Bacteria Cells in the Body." PLoS Biology 14 (8). <https://doi.org/10.1371/journal.pbio.1002533>.
3. www.evocell-itn.eu;
4. Macaulay, Iain C., and Thierry Voet. 2014. "Single Cell Genomics: Advances and Future Perspectives." PLOS Genetics 10 (1): e1004126. <https://doi.org/10.1371/journal.pgen.1004126>.
5. Pryor, Rosina, Povilas Norvaisas, Georgios Marinos, Lena Best, Louise B. Thingholm, Leonor M. Quintaneiro, Wouter De Haes, et al. 2019. "Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy." Cell 178 (6): 1299-1312.e29. <https://doi.org/10.1016/j.cell.2019.08.003>.
6. Cully, Megan. 2019. "Microbiome Therapeutics Go Small Molecule." *Nature Reviews Drug Discovery* 18 (July): 569. <https://doi.org/10.1038/d41573-019-00122-8>.
7. Duscha, Alexander, Barbara Gisevius, Sarah Hirschberg, Nissan Yissachar, Gabriele I. Stangl, Eva Eilers, Verian Bader, et al. 2020. "Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism." Cell 180 (6): 1067-1080.e16. <https://doi.org/10.1016/j.cell.2020.02.035>.
8. Pryor, Rosina, Povilas Norvaisas, Georgios Marinos, Lena Best, Louise B. Thingholm, Leonor M. Quintaneiro, Wouter De Haes, et al. 2019. "Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy." Cell 178 (6): 1299-1312.e29. <https://doi.org/10.1016/j.cell.2019.08.003>.
9. Zimmermann, Michael, Maria Zimmermann-Kogadeeva, Rebekka Wegmann, and Andrew L. Goodman. 2019. "Mapping Human Microbiome Drug Metabolism by Gut Bacteria and Their Genes." Nature 570 (7762): 462. <https://doi.org/10.1038/s41586-019-1291-3>.
10. Shin, Hyun Kil, Young-Mook Kang, and Kyoung Tai No. 2016. "Predicting ADME Properties of Chemicals." In *Handbook of Computational Chemistry*, edited by Jerzy Leszczynski, 1–37. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-6169-8_59-1.

References (continued)

11. Mädler, Sophia Clara, Alice Julien-Laferriere, Luis Wyss, Miroslav Phan, Albert S. W. Kang, Eric Ulrich, Roland Schmucki, et al. 2020. "Besca, a Single-Cell Transcriptomics Analysis Toolkit to Accelerate Translational Research." *BioRxiv*, September, 2020.08.11.245795. <https://doi.org/10.1101/2020.08.11.245795>.
12. Andrews, Tallulah S., Vladimir Yu Kiselev, Davis McCarthy, and Martin Hemberg. 2021. "Tutorial: Guidelines for the Computational Analysis of Single-Cell RNA Sequencing Data." *Nature Protocols* 16 (1): 1–9. <https://doi.org/10.1038/s41596-020-00409-w>.
13. Sturm, Gregor, Francesca Finotello, Florent Petitprez, Jitao David Zhang, Jan Baumbach, Wolf H. Fridman, Markus List, and Tatsiana Aneichyk. 2019. "Comprehensive Evaluation of Transcriptome-Based Cell-Type Quantification Methods for Immuno-Oncology." *Bioinformatics* 35 (14): i436–45. <https://doi.org/10.1093/bioinformatics/btz363>.
14. Villani, Alexandra-Chloé, Rahul Satija, Gary Reynolds, Siranush Sarkizova, Karthik Shekhar, James Fletcher, Morgane Griesbeck, et al. 2017. "Single-Cell RNA-Seq Reveals New Types of Human Blood Dendritic Cells, Monocytes, and Progenitors." *Science* 356 (6335): eaah4573. <https://doi.org/10.1126/science.aah4573>.
15. Finotello, Francesca, Clemens Mayer, Christina Plattner, Gerhard Laschober, Dietmar Rieder, Hubert Hackl, Anne Krogsdam, et al. 2019. "Molecular and Pharmacological Modulators of the Tumor Immune Contexture Revealed by Deconvolution of RNA-Seq Data." *Genome Medicine* 11 (1): 34. <https://doi.org/10.1186/s13073-019-0638-6>.
16. Fridman, Wolf H., Laurence Zitvogel, Catherine Sautès-Fridman, and Guido Kroemer. 2017. "The Immune Contexture in Cancer Prognosis and Treatment." *Nature Reviews Clinical Oncology* 14 (12): 717–34. <https://doi.org/10.1038/nrclinonc.2017.101>.
17. Moisan, Annie, Marcel Gubler, Jitao David Zhang, Yann Tessier, Kamille Dumong Erichsen, Sabine Sewing, Régine Gérard, et al. 2017. "Inhibition of EGF Uptake by Nephrotoxic Antisense Drugs In Vitro and Implications for Preclinical Safety Profiling." *Molecular Therapy - Nucleic Acids* 6 (March): 89–105. <https://doi.org/10.1016/j.omtn.2016.11.006>.
18. Chang, Chia-Yu, Hsiao-Chien Ting, Ching-Ann Liu, Hong-Lin Su, Tzyy-Wen Chiou, Horng-Jyh Harn, and Shinn-Zong Lin. 2018. "Induced Pluripotent Stem Cells: A Powerful Neurodegenerative Disease Modeling Tool for Mechanism Study and Drug Discovery." *Cell Transplantation* 27 (June): 096368971877540. <https://doi.org/10.1177/0963689718775406>.

References (continued)

19. Takahashi, Toshio. 2019. "Organoids for Drug Discovery and Personalized Medicine." *Annual Review of Pharmacology and Toxicology* 59 (1): 447–62. <https://doi.org/10.1146/annurev-pharmtox-010818-021108>.
20. Budayeva, Hanna G., and Donald S. Kirkpatrick. 2020. "Monitoring Protein Communities and Their Responses to Therapeutics." *Nature Reviews Drug Discovery* 19 (6): 414–26. <https://doi.org/10.1038/s41573-020-0063-y>.
21. Lukonin, Ilya, Denise Serra, Ludivine Challet Meylan, Katrin Volkmann, Janine Baaten, Rui Zhao, Shelly Meeusen, et al. 2020. "Phenotypic Landscape of Intestinal Organoid Regeneration." *Nature* 586 (7828): 275–80. <https://doi.org/10.1038/s41586-020-2776-9>.
22. Drawnel, Faye M., Stefano Boccardo, Michael Prummer, Frédéric Delobel, Alexandra Graff, Michael Weber, Régine Gérard, et al. 2014. "Disease Modeling and Phenotypic Drug Screening for Diabetic Cardiomyopathy Using Human Induced Pluripotent Stem Cells." *Cell Reports* 9 (3): 810–20. <https://doi.org/10.1016/j.celrep.2014.09.055>.
23. Traag, Vincent, Ludo Waltman, and Nees Jan van Eck. 2019. "From Louvain to Leiden: Guaranteeing Well-Connected Communities." *Scientific Reports* 9 (1): 5233. <https://doi.org/10.1038/s41598-019-41695-z>.
24. *Understanding UMAP*, Andy Coenen and Adam Pearce, <https://pair-code.github.io/understanding-umap/>
25. How exactly UMAP works, Nikolay Oskolkov, <https://towardsdatascience.com/how-exactly-umap-works-13e3040e1668>
26. McInnes, Leland, and John Healy. 2018. "UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction." ArXiv:1802.03426 [Cs, Stat], February. <http://arxiv.org/abs/1802.03426>.
27. Zappia, Luke, Belinda Phipson, and Alicia Oshlack. 2018. "Exploring the Single-Cell RNA-Seq Analysis Landscape with the ScRNA-Tools Database." *PLOS Computational Biology* 14 (6): e1006245. <https://doi.org/10.1371/journal.pcbi.1006245>.
28. Abdelaal, Tamim, Lieke Michielsen, Davy Cats, Dylan Hoogduin, Hailiang Mei, Marcel J. T. Reinders, and Ahmed Mahfouz. 2019. "A Comparison of Automatic Cell Identification Methods for Single-Cell RNA Sequencing Data." *Genome Biology* 20 (1): 194. <https://doi.org/10.1186/s13059-019-1795-z>.
29. Janas, Maja M., Mark K. Schlegel, Carole E. Harbison, Vedat O. Yilmaz, Yongfeng Jiang, Rubina Parmar, Ivan Zlatev, et al. 2018. "Selection of GalNAc-Conjugated SiRNAs with Limited off-Target-Driven Rat Hepatotoxicity." *Nature Communications* 9 (1): 723. <https://doi.org/10.1038/s41467-018-02989-4>.

References (continued)

30. Jackson, Aimee L., and Peter S. Linsley. 2010. "Recognizing and Avoiding SiRNA Off-Target Effects for Target Identification and Therapeutic Application." *Nature Reviews Drug Discovery* 9 (1): 57–67. <https://doi.org/10.1038/nrd3010>.
31. Romond, Edward H., Edith A. Perez, John Bryant, Vera J. Suman, Charles E. Geyer, Nancy E. Davidson, Elizabeth Tan-Chiu, et al. 2005. "Trastuzumab plus Adjuvant Chemotherapy for Operable HER2-Positive Breast Cancer." *New England Journal of Medicine* 353 (16): 1673–84. <https://doi.org/10.1056/NEJMoa052122>.
32. Gao, Jinxu, Adelphe Mfuh, Yuka Amako, and Christina M. Woo. 2018. "Small Molecule Interactome Mapping by Photoaffinity Labeling Reveals Binding Site Hotspots for the NSAIDs." *Journal of the American Chemical Society* 140 (12): 4259–68. <https://doi.org/10.1021/jacs.7b11639>.
33. Bollag, Gideon, James Tsai, Jiazhong Zhang, Chao Zhang, Prabha Ibrahim, Keith Nolop, and Peter Hirth. 2012. "Vemurafenib: The First Drug Approved for BRAF -Mutant Cancer." *Nature Reviews Drug Discovery* 11 (11): 873–86. <https://doi.org/10.1038/nrd3847>.
34. Luebker, Stephen A., and Scott A. Koepsell. 2019. "Diverse Mechanisms of BRAF Inhibitor Resistance in Melanoma Identified in Clinical and Preclinical Studies." *Frontiers in Oncology* 9. <https://doi.org/10.3389/fonc.2019.00268>.
35. Kimball's Biology Page, <http://www.biology-pages.info/>
36. Molina, Daniel Martinez, Rozbeh Jafari, Marina Ignatushchenko, Takahiro Seki, E. Andreas Larsson, Chen Dan, Lekshmy Sreekumar, Yihai Cao, and Pär Nordlund. 2013. "Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay." *Science* 341 (6141): 84–87. <https://doi.org/10.1126/science.1233606>.
37. Zhou, Zheng, Taishan Hu, Xue Zhou, Steffen Wildum, Fernando Garcia-Alcalde, Zhiheng Xu, Daitze Wu, et al. 2017. "Heteroaryldihydropyrimidine (HAP) and Sulfamoylbenzamide (SBA) Inhibit Hepatitis B Virus Replication by Different Molecular Mechanisms." *Scientific Reports* 7 (1): 42374. <https://doi.org/10.1038/srep42374>.
38. Dai, Jiang, Yi-Jiao Huang, Xinhua He, Ming Zhao, Xinzhen Wang, Zhao-Shan Liu, Wen Xue, et al. 2019. "Acetylation Blocks CGAS Activity and Inhibits Self-DNA-Induced Autoimmunity." *Cell* 176 (6): 1447–1460.E14. <https://doi.org/10.1016/j.cell.2019.01.016>.

References (continued)

39. Hart, Charles P. 2005. "Finding the Target after Screening the Phenotype." *Drug Discovery Today* 10 (7): 513–19.
[https://doi.org/10.1016/S1359-6446\(05\)03415-X](https://doi.org/10.1016/S1359-6446(05)03415-X).
40. Ziegler, Slava, Sonja Sievers, and Herbert Waldmann. 2021. "Morphological Profiling of Small Molecules." *Cell Chemical Biology* 28 (3): 300–319.
<https://doi.org/10.1016/j.chembiol.2021.02.012>.
41. Winter, Georg E., Dennis L. Buckley, Joshiawa Paulk, Justin M. Roberts, Amanda Souza, Sirano Dhe-Paganon, and James E. Bradner. 2015. "Phthalimide Conjugation as a Strategy for in Vivo Target Protein Degradation." *Science* 348 (6241): 1376–81.
<https://doi.org/10.1126/science.aab1433>.
42. Aebersold, Ruedi, and Matthias Mann. 2016. "Mass-Spectrometric Exploration of Proteome Structure and Function." *Nature* 537 (7620): 347–55.
<https://doi.org/10.1038/nature19949>.
43. Zhou, Jing C., Bob Feller, Bill Hinsberg, Geeta Sethi, Paul Feldstein, Joshua Hihath, Erkin Seker, Maria Marco, Andre Knoesen, and Robert Miller. 2015. "Immobilization-Mediated Reduction in Melting Temperatures of DNA–DNA and DNA–RNA Hybrids: Immobilized DNA Probe Hybridization Studied by SPR." *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 481 (September): 72–79.
<https://doi.org/10.1016/j.colsurfa.2015.04.046>.
44. Hagedorn, Peter H., Malene Pontoppidan, Tina S. Bisgaard, Marco Berrera, Andreas Dieckmann, Martin Ebeling, Marianne R. Møller, et al. 2018. "Identifying and Avoiding Off-Target Effects of RNase H-Dependent Antisense Oligonucleotides in Mice." *Nucleic Acids Research* 46 (11): 5366–80.
<https://doi.org/10.1093/nar/gky397>.
45. Rehmsmeier, Marc, Peter Steffen, Matthias Hochsmann, and Robert Giegerich. 2004. "Fast and Effective Prediction of MicroRNA/Target Duplexes." *RNA* (New York, N.Y.) 10 (10): 1507–17. <https://doi.org/10.1261/rna.5248604>.
46. Tyanova, Stefka, Tikira Temu, and Juergen Cox. 2016. "The MaxQuant Computational Platform for Mass Spectrometry-Based Shotgun Proteomics." *Nature Protocols* 11 (12): 2301–19. <https://doi.org/10.1038/nprot.2016.136>.
47. xkcd: <https://xkcd.com/1217/>

References (continued)

48. Murin, Charles D. "Considerations of Antibody Geometric Constraints on NK Cell Antibody Dependent Cellular Cytotoxicity." *Frontiers in Immunology* 11 (2020). <https://www.frontiersin.org/article/10.3389/fimmu.2020.01635>.
49. Marx, Vivien. "Method of the Year: Spatially Resolved Transcriptomics." *Nature Methods* 18, no. 1 (January 2021): 9–14. <https://doi.org/10.1038/s41592-020-01033-y>.
50. Andrade, E. L., A. F. Bento, J. Cavalli, S. K. Oliveira, R. C. Schwanke, J. M. Siqueira, C. S. Freitas, R. Marcon, and J. B. Calixto. "Non-Clinical Studies in the Process of New Drug Development - Part II: Good Laboratory Practice, Metabolism, Pharmacokinetics, Safety and Dose Translation to Clinical Studies." *Brazilian Journal of Medical and Biological Research* 49 (December 12, 2016). <https://doi.org/10.1590/1414-431X20165646>.
51. Olson, H., G. Betton, D. Robinson, K. Thomas, A. Monro, G. Kolaja, P. Lilly, et al. "Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals." *Regulatory Toxicology and Pharmacology: RTP* 32, no. 1 (August 2000): 56–67. <https://doi.org/10.1006/rtpb.2000.1399>.
52. Tamaki, Chihiro, Takashi Nagayama, Masamichi Hashiba, Masato Fujiyoshi, Masanori Hizue, Hiroshi Kodaira, Minoru Nishida, et al. "Potentials and Limitations of Nonclinical Safety Assessment for Predicting Clinical Adverse Drug Reactions: Correlation Analysis of 142 Approved Drugs in Japan." *The Journal of Toxicological Sciences* 38, no. 4 (2013): 581–98. <https://doi.org/10.2131/jts.38.581>.
53. Burton, Nikolas R., Phillip Kim, and Keriann M. Backus. "Photoaffinity Labelling Strategies for Mapping the Small Molecule–Protein Interactome." *Organic & Biomolecular Chemistry* 19, no. 36 (September 22, 2021): 7792–7809. <https://doi.org/10.1039/D1OB01353J>.
54. Zecha, Jana, Florian P. Bayer, Svenja Wiechmann, Julia Woortman, Nicola Berner, Julian Müller, Annika Schneider, et al. "Decrysting Drug Actions and Protein Modifications by Dose- and Time-Resolved Proteomics." *Science* 380, no. 6640 (April 7, 2023): 93–101. <https://doi.org/10.1126/science.adc3925>.
55. Audagnotto, Martina, and Matteo Dal Peraro. "Protein Post-Translational Modifications: In Silico Prediction Tools and Molecular Modeling." *Computational and Structural Biotechnology Journal* 15 (January 1, 2017): 307–19.

References (continued)

56. Perkel, J. M. 85 million cells — and counting — at your fingertips. *Nature* 629, 248–249 (2024).
57. Lotfollahi, M., Hao, Y., Theis, F. J. & Satija, R. The future of rapid and automated single-cell data analysis using reference mapping. *Cell* 187, 2343–2358 (2024).

Supplementary Information

Embryonic origins of tissues

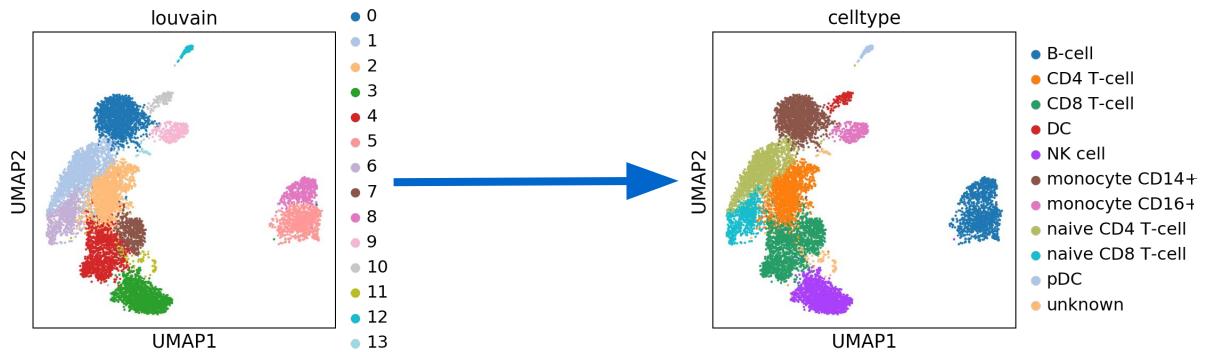
Germ Layer	Gives rise to:		
Ectoderm	Epidermis, glands on skin, some cranial bones, pituitary and adrenal medulla, the nervous system, the mouth between cheek and gums, the anus		
Mesoderm	Connective tissues proper, bone, cartilage, blood, endothelium of blood vessels, muscle, synovial membranes, serous membranes lining body cavities, kidneys, lining of gonads		
Endoderm	Lining of airways and digestive system except the mouth and distal part of digestive system (rectum and anal canal); glands (digestive glands, endocrine glands, adrenal cortex)		

An intern project: Cell type annotation

From unsupervised clustering and cluster based annotation



Luis Wyss
RAAN intern 2019



	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Label
Training Cell 1	10	50	0	12	4	Celltype A
Training Cell 2	8	45	78	3	23	Celltype B
Training Cell 3	14	55	78	65	55	Celltype B
Training Cell 4	78	12	13	9	58	Celltype A
Training Cell 5	45	23	65	98	11	Celltype C

To supervised annotation at single-cell level:

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
Cell 1	45	45	8	56	3
Cell 2	65	120	78	45	12
Cell 3	79	12	34	65	88
Cell 4	7	59	32	47	62

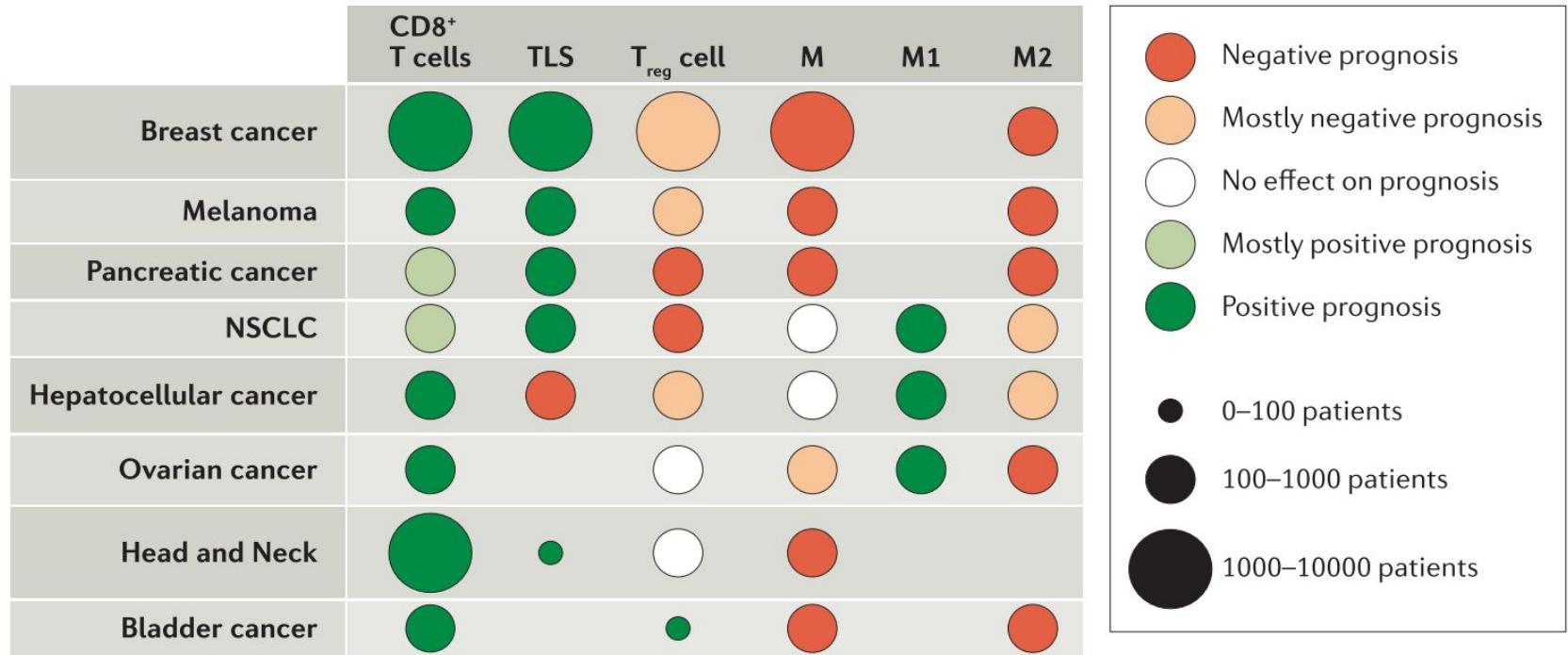


	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Prediction
Cell 1	45	45	8	56	3	Celltype A
Cell 2	65	120	78	45	12	Celltype B
Cell 3	79	12	34	65	88	Celltype C
Cell 4	7	59	32	47	62	Celltype B



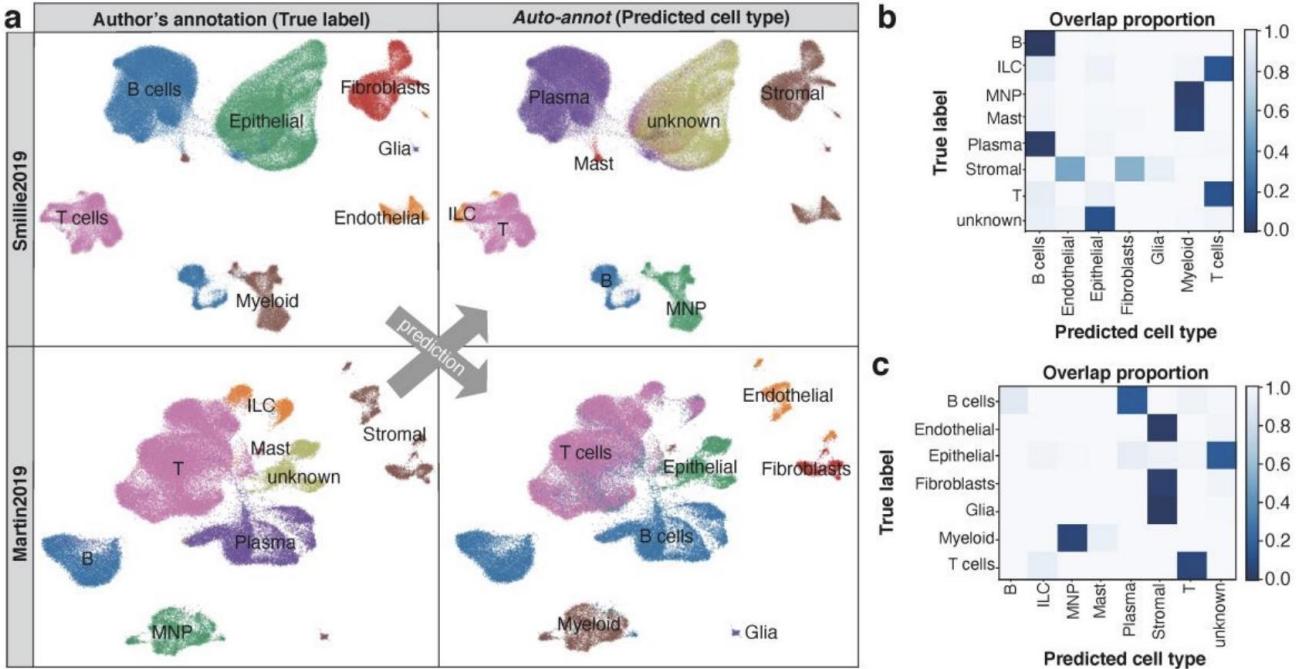
Advantages: (1) automation, (2) annotation independent from clustering, and (3) we can estimate the confidence of prediction

Abundance of immune cells in tumor microenvironments affect outcome



TLS: tertiary lymphoid structures; T_{reg}: regulatory T cells; M: macrophages; M1/M2: subtypes of macrophages

An example of Inflammatory Bowel Disease (IBD)



We observed Inconsistent cell type nomenclature across studies.
 Machine learning allows us compare and integrate multiple studies.

We are living ecosystems

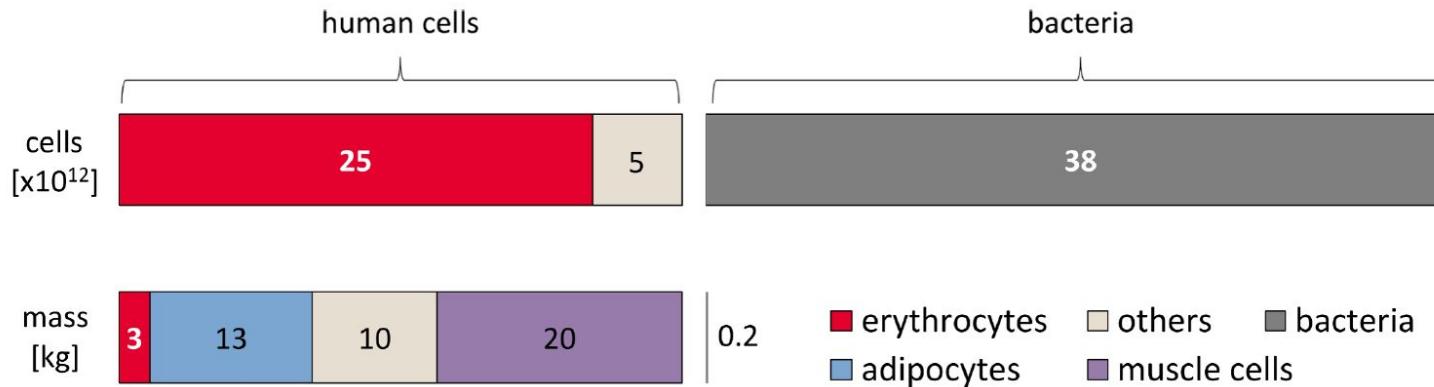
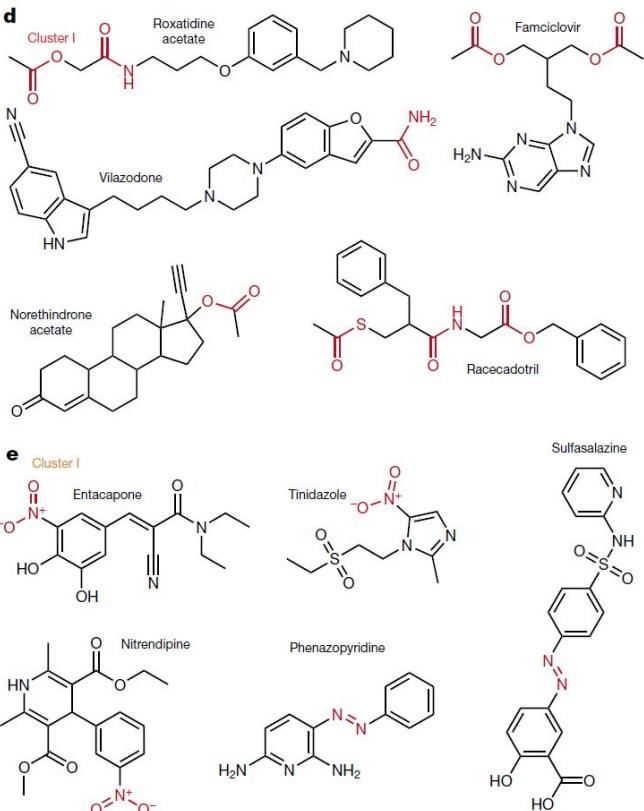
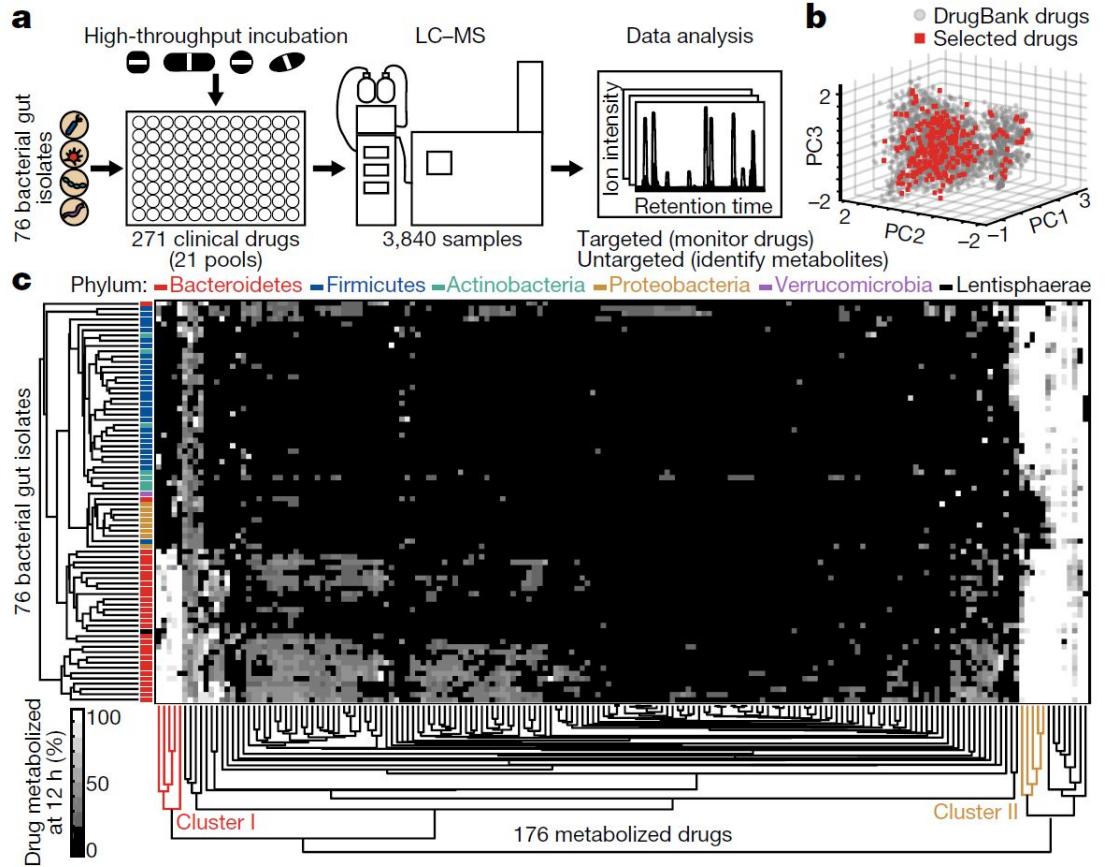


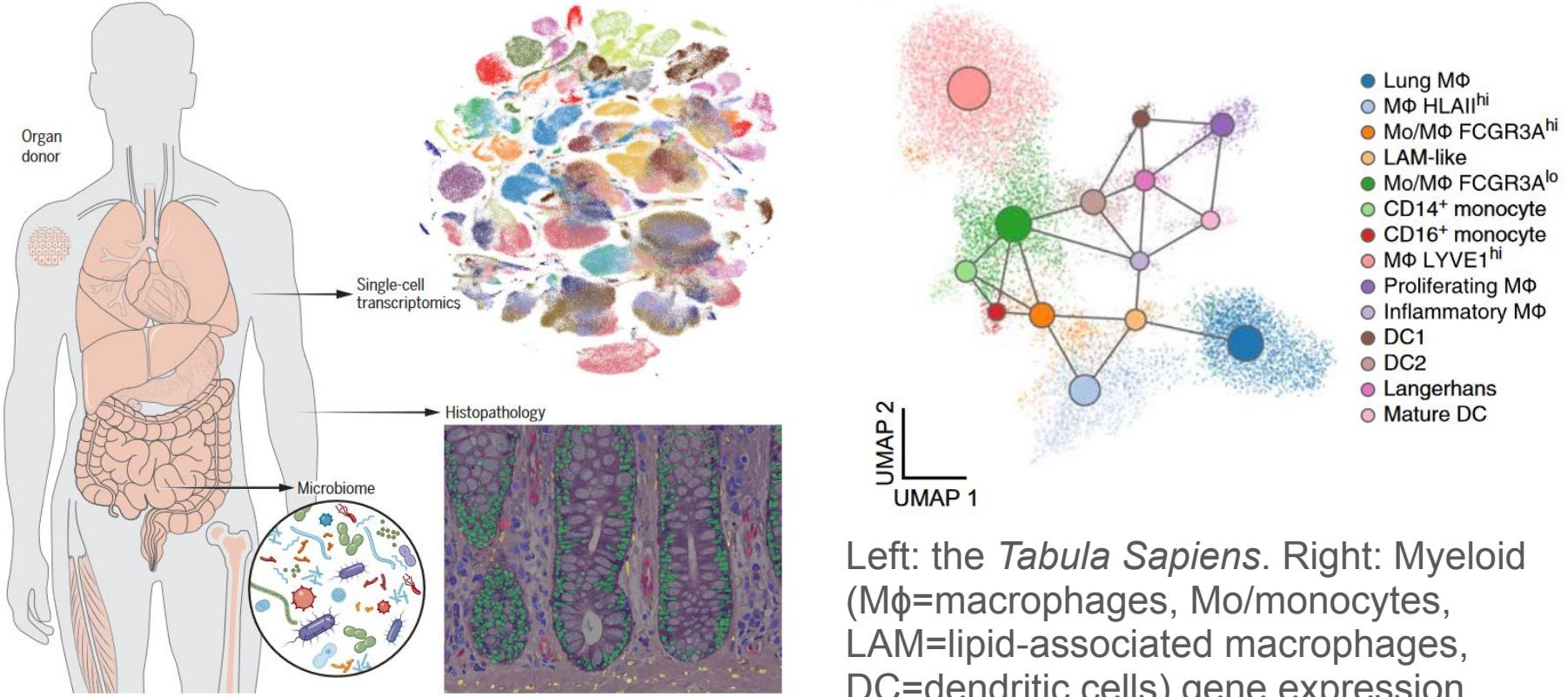
Table 3. B/H ratio for different population. See Table B in [S1 Appendix](#) for full references.

population segment	body weight [kg]	age [y]	blood volume [L]	RBC count [$10^{12}/\text{L}$]	colon content [g]	bac. conc. [$10^{11}/\text{g wet}$] ⁽¹⁾	total human cells [10^{12}] ⁽²⁾	total bacteria [10^{12}] ⁽²⁾	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	1.3
ref. woman	63		3.9	4.5	480	0.92	21	44	2.2
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	2.3
infant	9.6	1	0.8	4.5	80	0.92	4	7	1.7
elder	70	66	3.8 ⁽³⁾	4.8	420	0.92	22	38	1.8
obese	140		6.7	5.0 ⁽⁴⁾	610 ⁽⁵⁾	0.92	40	56	1.4

Gut microbiome can metabolize drugs differently



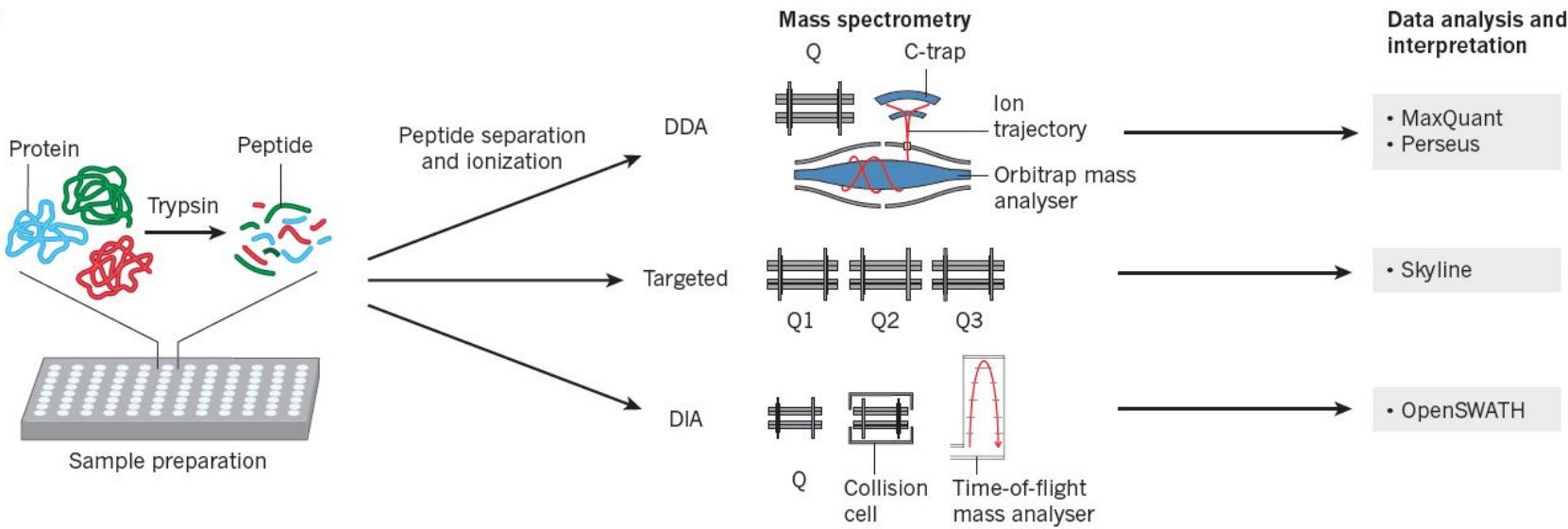
The *Tabula Sapiens* and other community projects offer reference expression data in healthy donors



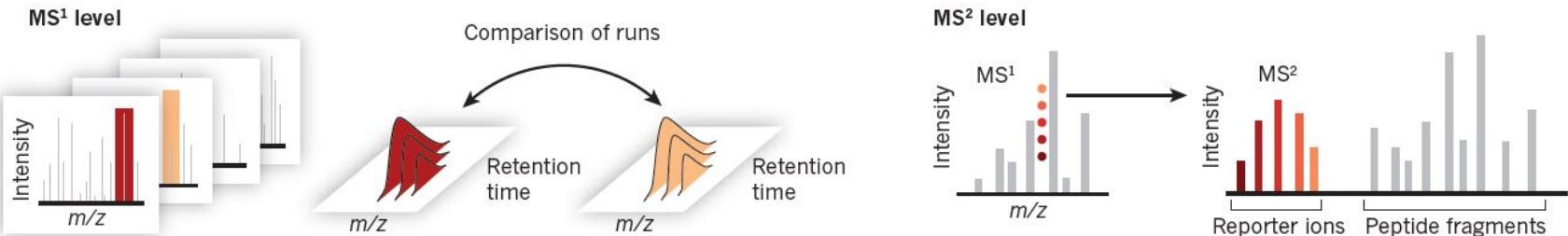
Left: the *Tabula Sapiens*. Right: Myeloid (MΦ=macrophages, Mo/monocytes, LAM=lipid-associated macrophages, DC=dendritic cells) gene expression

Mass-spectrometry based proteomics

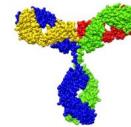
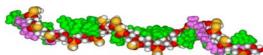
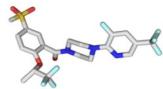
a



b Peptide quantification

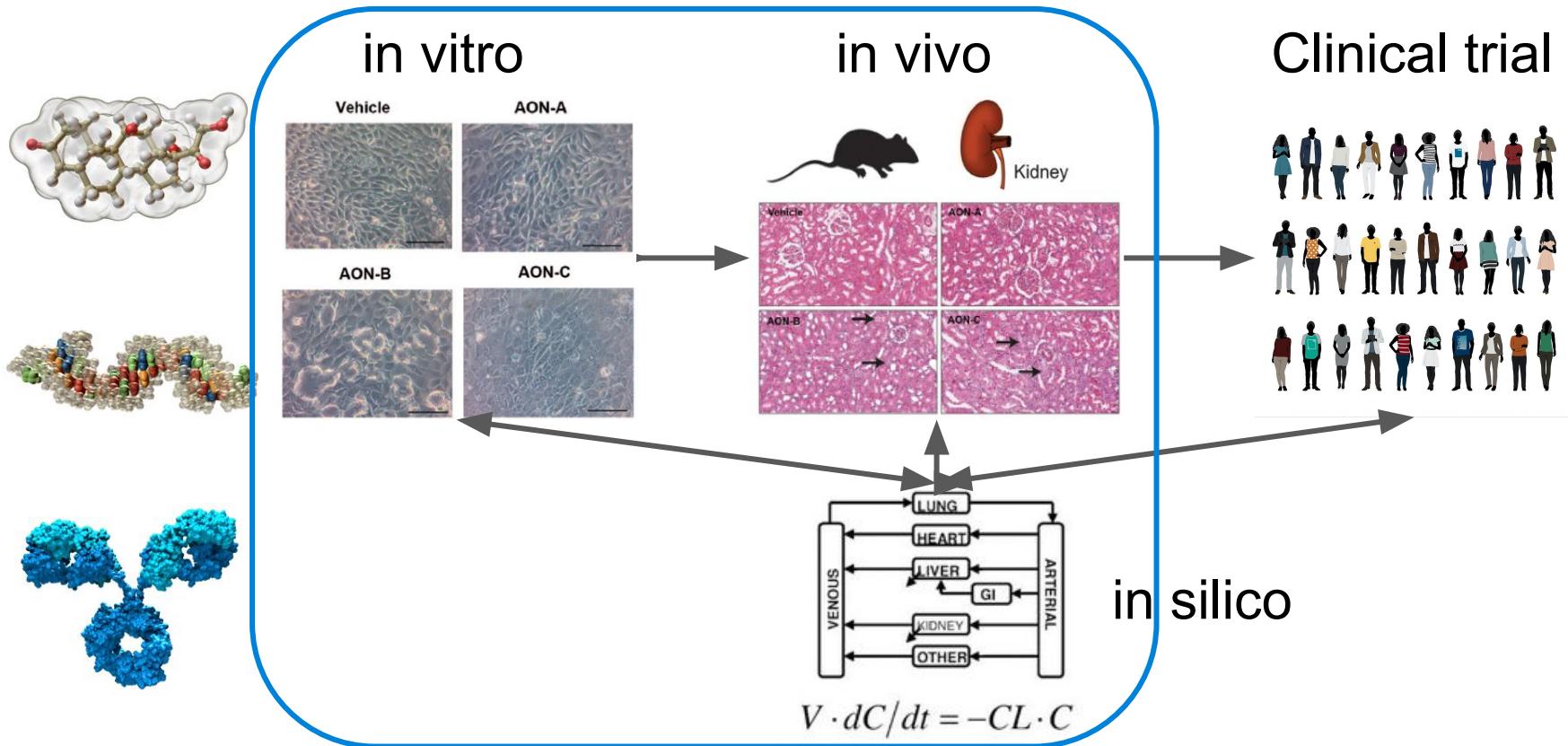


Comparing modalities with regard to safety assessment



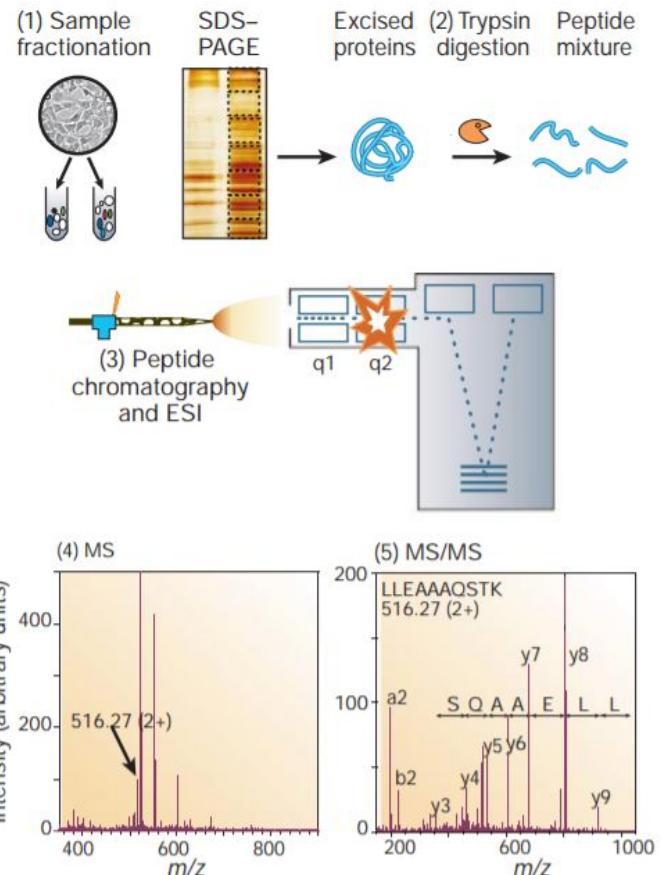
	Small molecules	Single Stranded Oligos	Biologics
Molecular weight	<1000 D	5000-7000 D	> 30000 D
Manufacture	Chemical synthesis	Chemical synthesis	Biologically-derived
Structure	Single entity, high purity	Single entity with 10-15% product-related impurities	Complex, heterogeneous
Chemical-driven toxicity	Yes	Yes	No
Metabolism	Species-specific	Species-independent catabolism by proteolytic degradation	Species-independent catabolism by proteolytic degradation
PK	Generally short $t_{1/2}$	Long (tissue) $t_{1/2}$	Long $t_{1/2}$
Some general aspects	High throughput screening/early safety testing of up to 500 small molecules	Biodistribution with consistent patterns	Fewer, yet complex due to biology/immunology

Proteomics plays an important role in *in vitro/in vivo* translation



Mass-spectrometry based proteomics

- **SDS-PAGE:** Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
- **ESI:** Electrospray ionization
- **q1/q2:** selection/collision/separation cells
- **MS:** Mass spectrometry
- **MS/MS:** tandem mass spectrometry



Proteomics approaches for drug discovery

Affinity purification

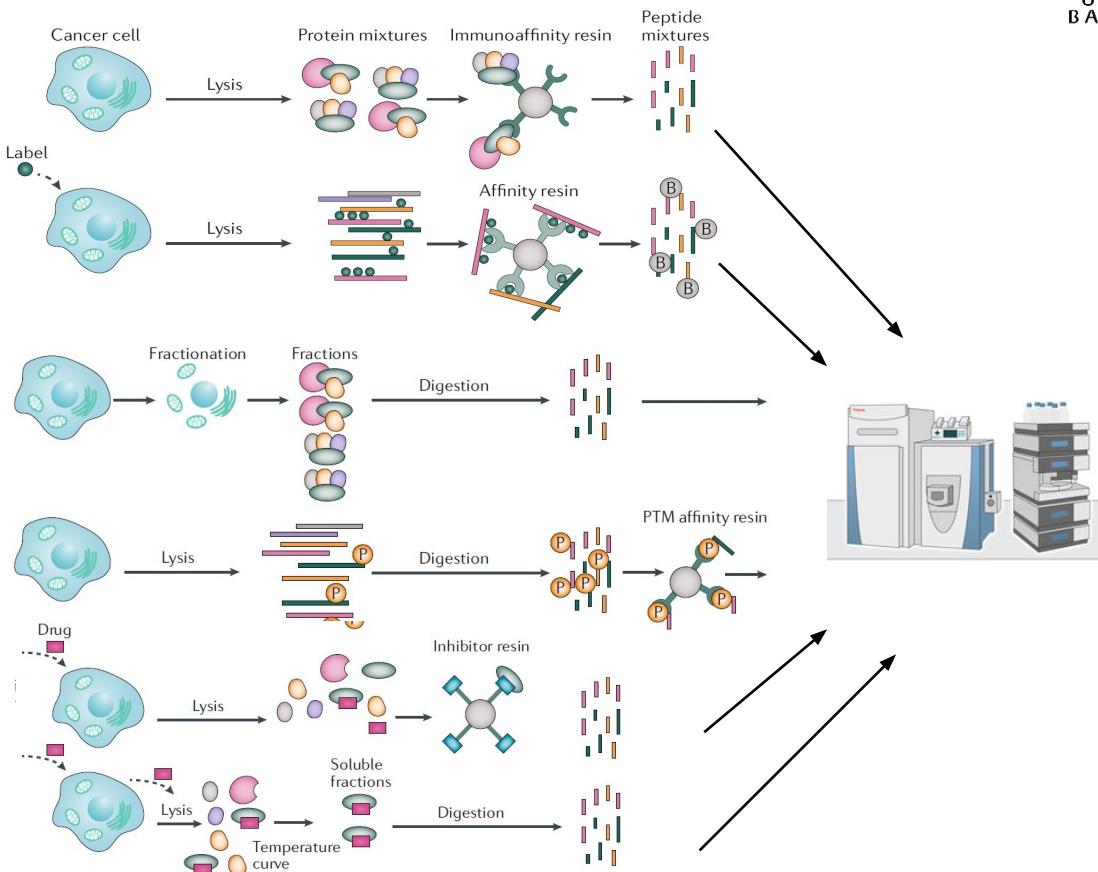
Proximity labelling

Organelle proteome profiling

Post-translational modification (PTM) profiling

Chemoaffinity enrichment

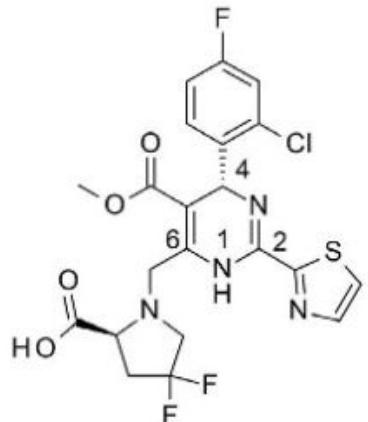
Thermal proteome profiling



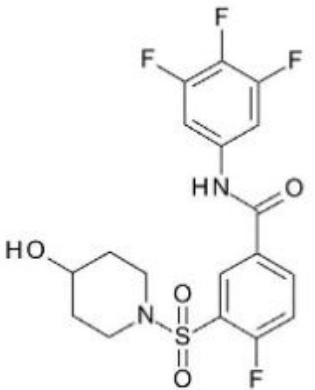
Case 1: Differentiate two compounds that inhibit Hepatitis B Virus with similar mode of action

a

HAP_R01

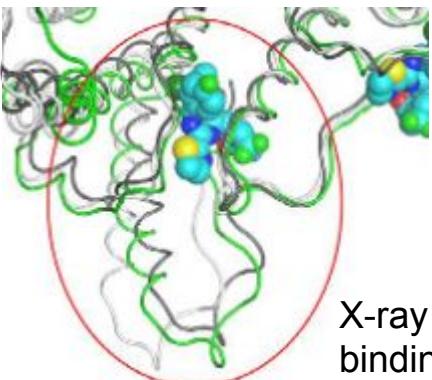


SBA_R01

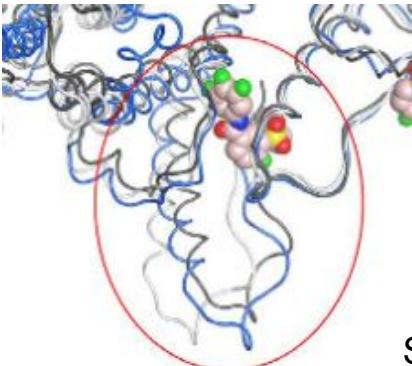


b

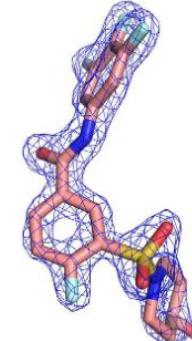
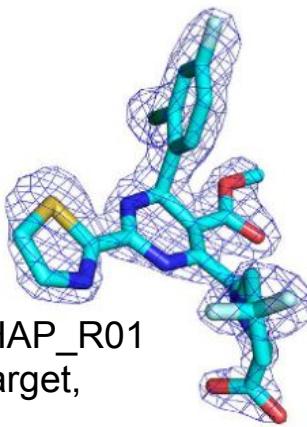
Compound	IC ₅₀ (μ M)	HepG2.2.15 EC ₅₀ (μ M)	CC ₅₀ (μ M)
HAP_R01	0.39 \pm 0.13	0.0064 \pm 0.0006	34.8 \pm 1.8
SBA_R01	1.90 \pm 0.22	0.26 \pm 0.02	8.05 \pm 0.92



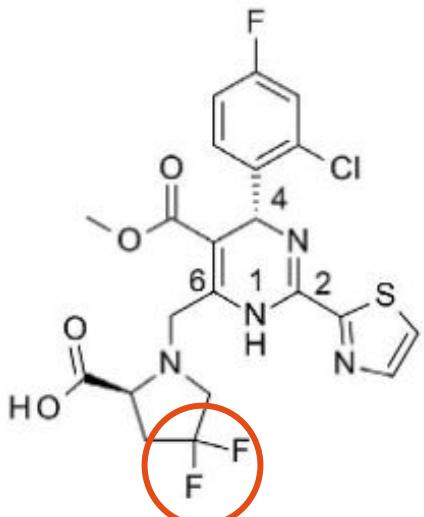
X-ray data of HAP_R01 binding to its target, HBV capsid



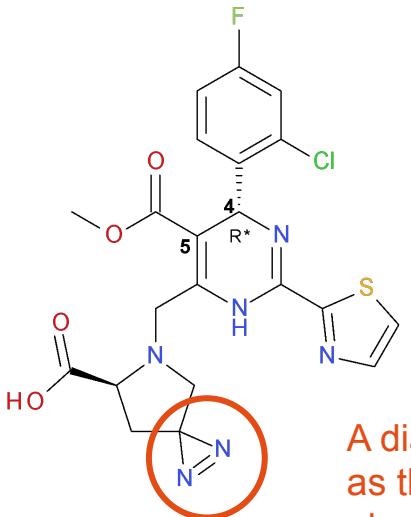
SBA_R01



Chemical probes: drug-like molecules to probe its mode of action

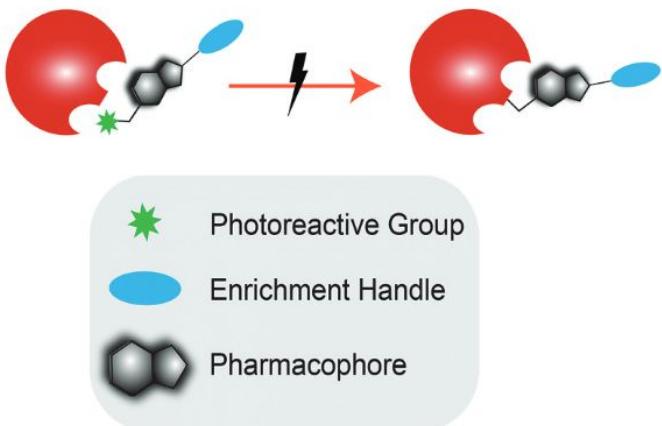


	IC_{50} (μM)
HAP_R01	0.39 ± 0.13

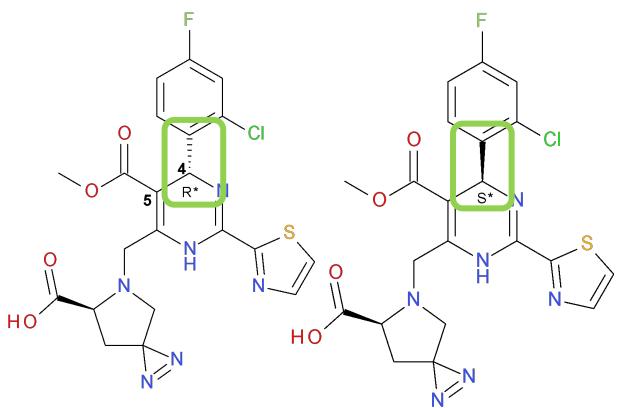


EC_{50} : **0.040 μM**
 IC_{50} : **0.47 μM**

A diazirine group
as the
photoreactive
group



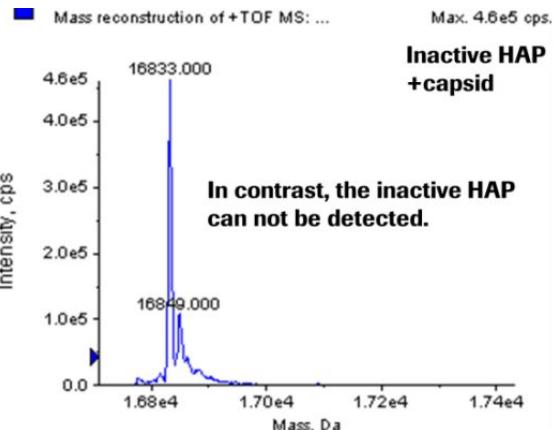
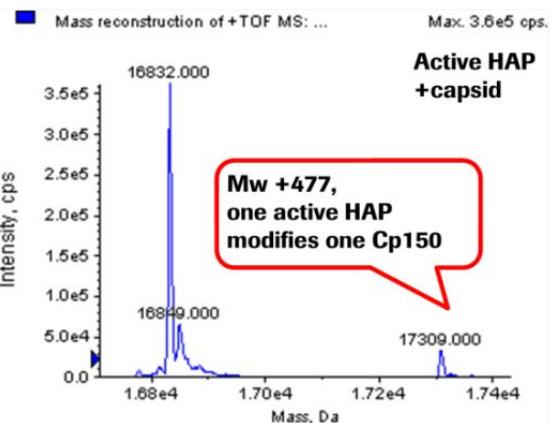
Case 1 solved: Proteomics confirmed target binding and mapped the small molecule binding pocket



RO-A
 EC_{50} : 0.040 μM
 IC_{50} : 0.47 μM

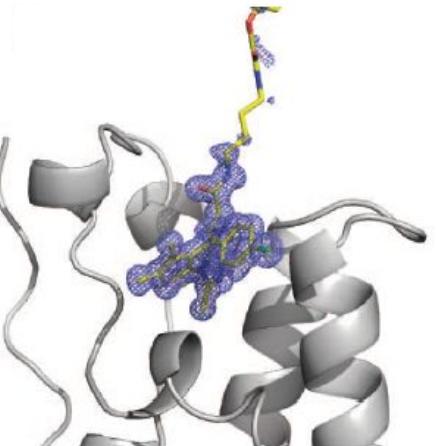
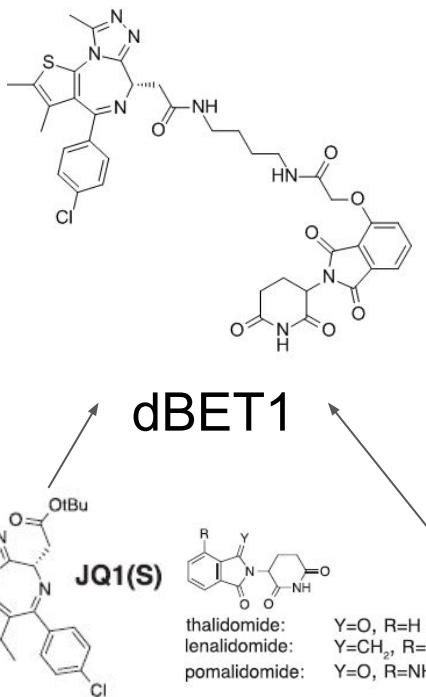
RO-B
 EC_{50} : >1 μM
 IC_{50} : >100 μM

+Cp150, UV, MS

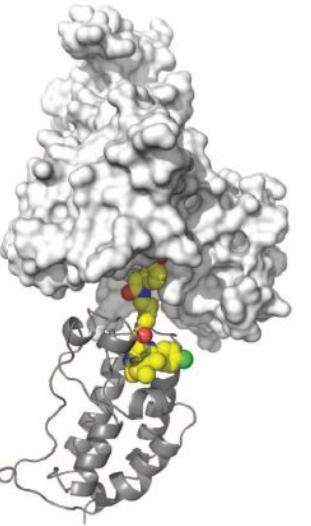


Proteolytic digestion/LC-MS/MS identified labelling site **Y118 (Y=Tyrosine)** of HBV capsid protein. More photoaffinity probes identified labelling sites at **R127 (R=Arginine)** and **Y38**.

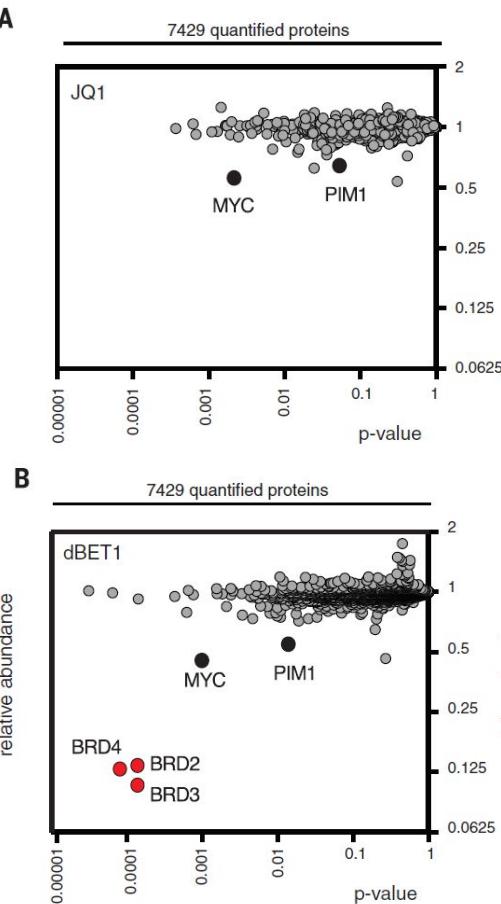
Case 2: Confirmation of selective degradation of protein target *in vivo*



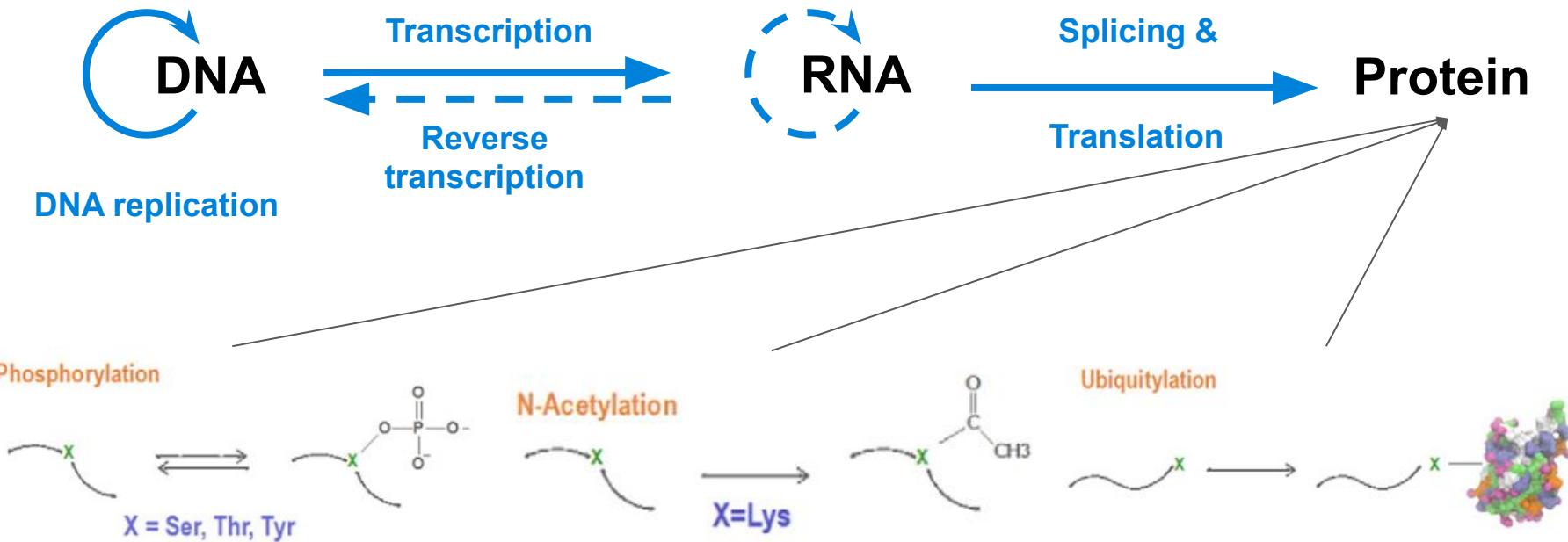
Crystal structure of dBET1 binding to its target BRD4



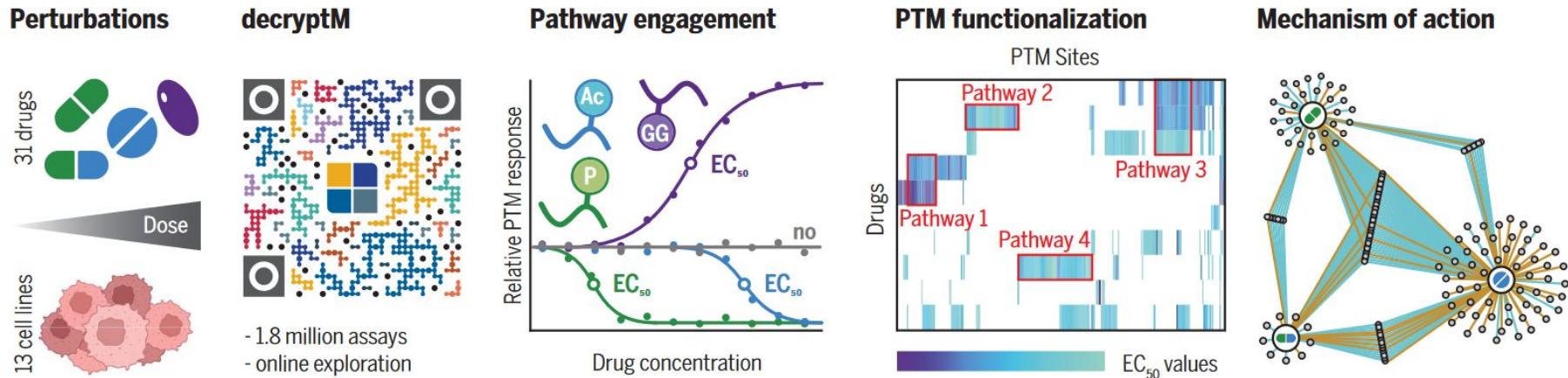
Docking of dBET1-BRD4 to DDB1-CRBN structure



Protein post-translational modifications (PTMs) offer an additional layer of regulation

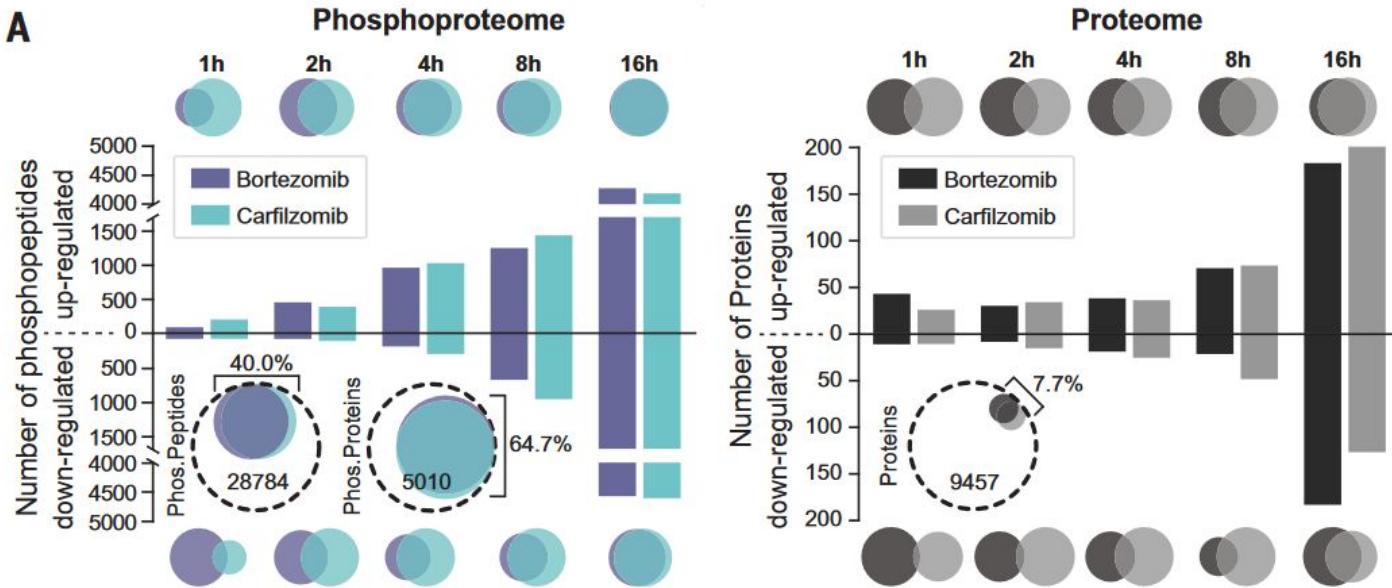


Case 3: Millions of PTM profiles induced by drugs in cancer cell lines



decryptM (Nature 2023): Following the dose-dependent treatment of cancer cells with drugs, quantitative mass spectrometry records dose-response of thousands of posttranslationally modified peptides. EC₅₀: half-maximal effective concentration; Ac, acetylation; GG, ubiquitinylation; P, phosphorylation.

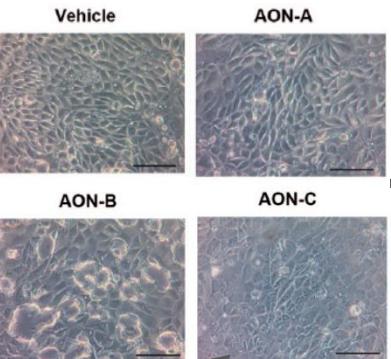
PTM and proteomics characterize MoA of drugs



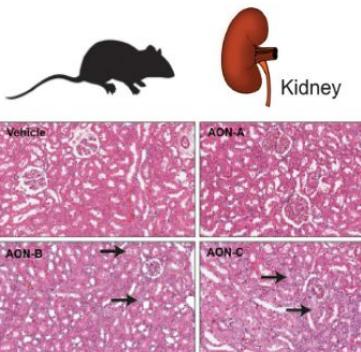
Bortezomib (BTZ) and carfilzomib (CFZ) both treat multiple myeloma by inhibiting the proteasome by reversible covalent (BTZ) or irreversible (CFZ) binding to the protease PSMB5. Time-series data show both the dynamics and the converging signaling.

Dose prediction based on pharmacology and toxicology before entry into human

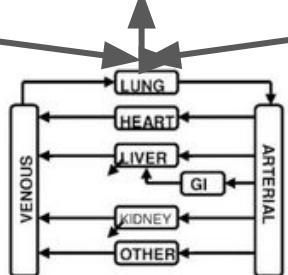
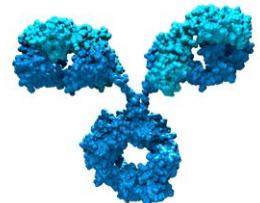
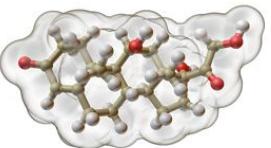
in vitro



in vivo



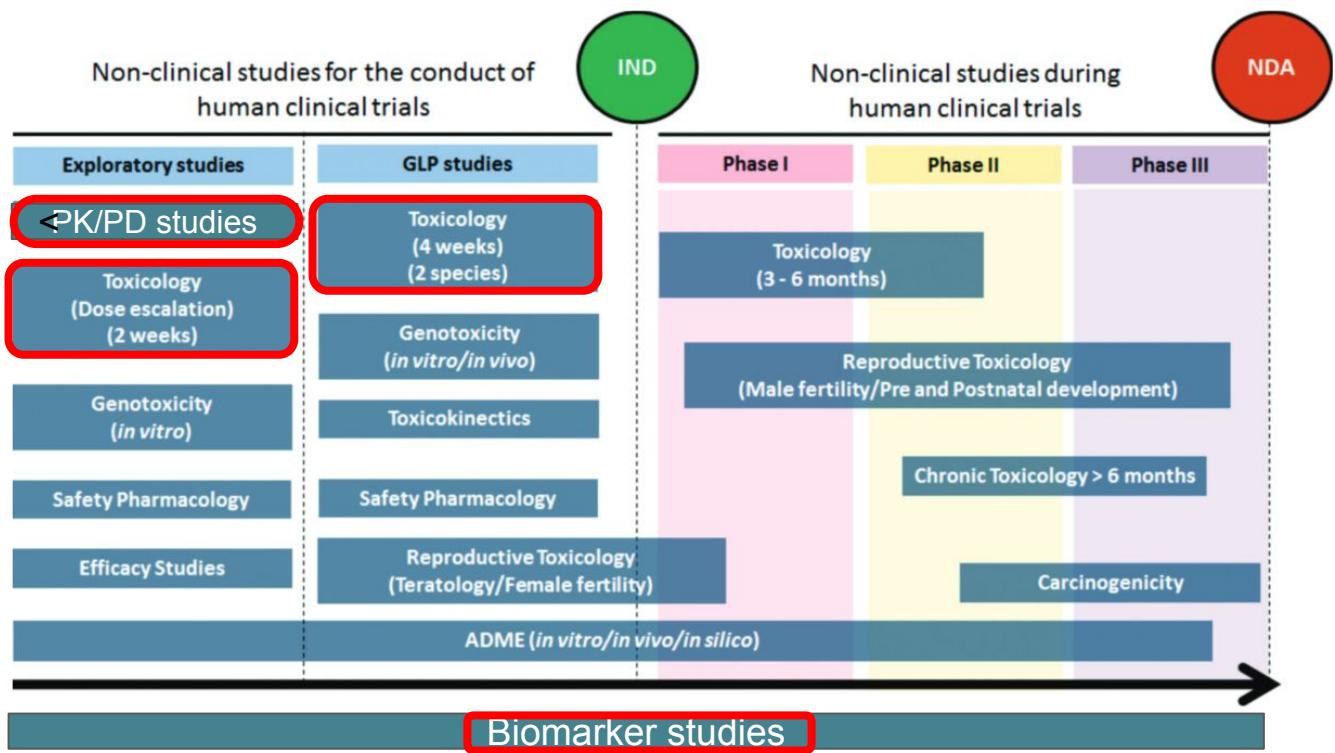
Clinical trial



in silico

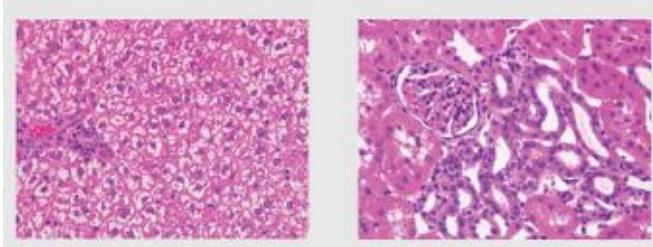
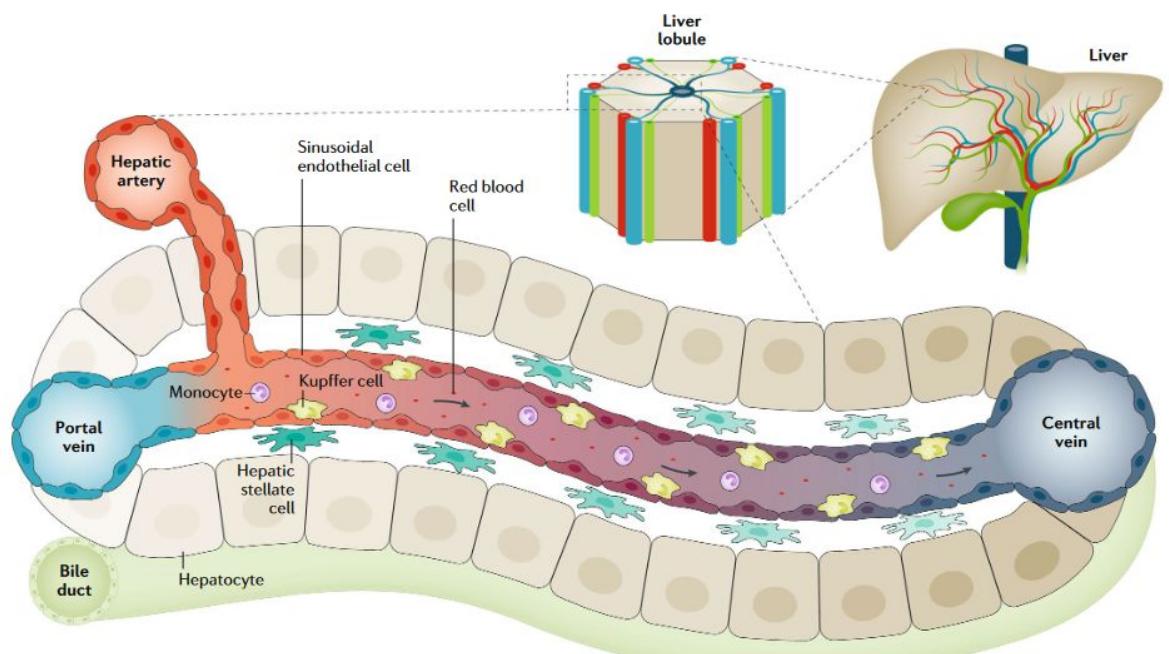
$$V \cdot dC/dt = -CL \cdot C$$

Current practices of non-clinical studies in drug development

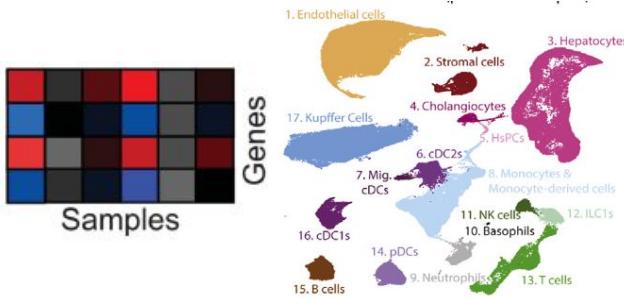


- IND: Investigational New Drug application
- NDA: New Drug Application
- GLP: Good Lab Practice
- Red boxes: Focus areas of this and coming lectures

Current practices of profiling and understanding toxicology: an example with liver



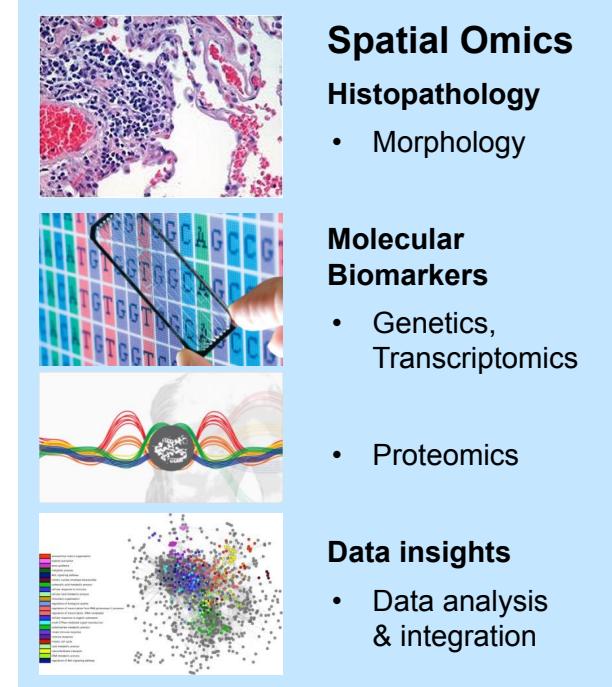
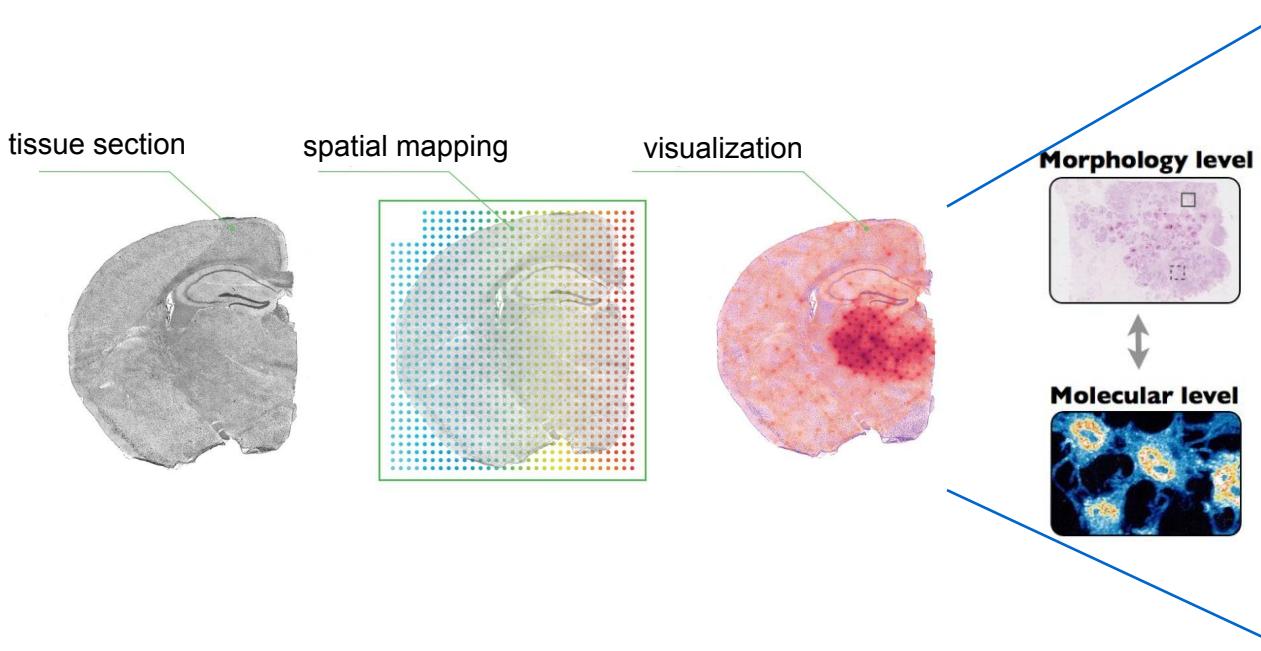
Histopathology



Omics

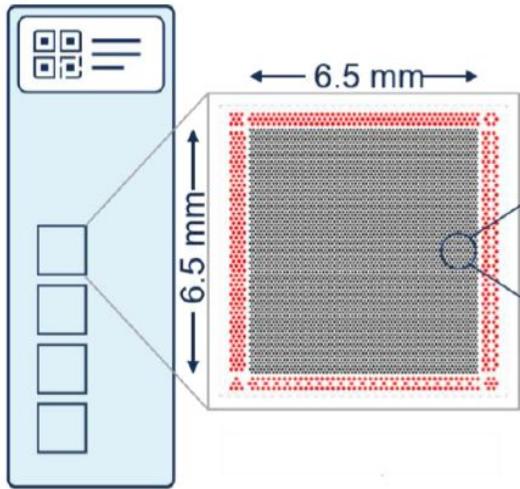
[Liver structure and anatomy \(YouTube Video\)](#)

Spatially resolved omics complement histopathology

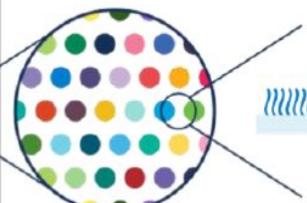


An example: 10x VISIUM Technology

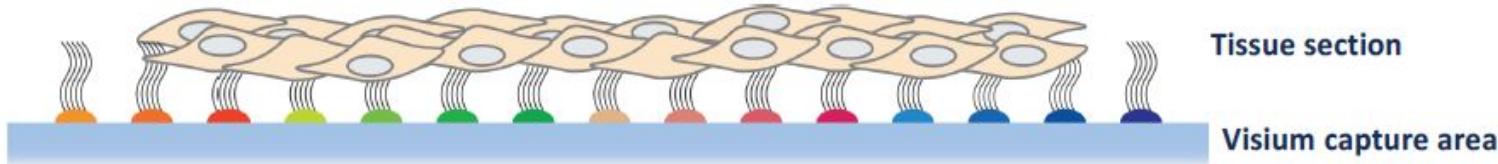
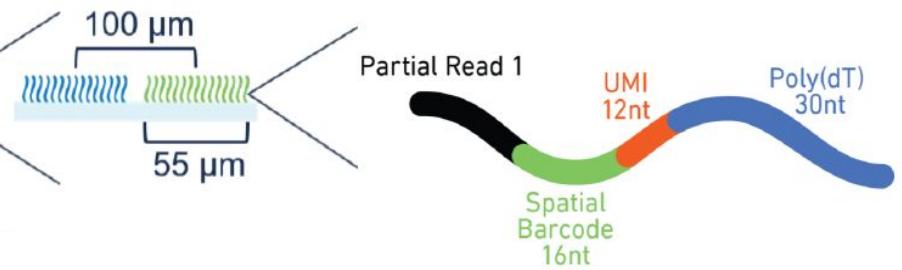
Visium Spatial Gene Expression Slide



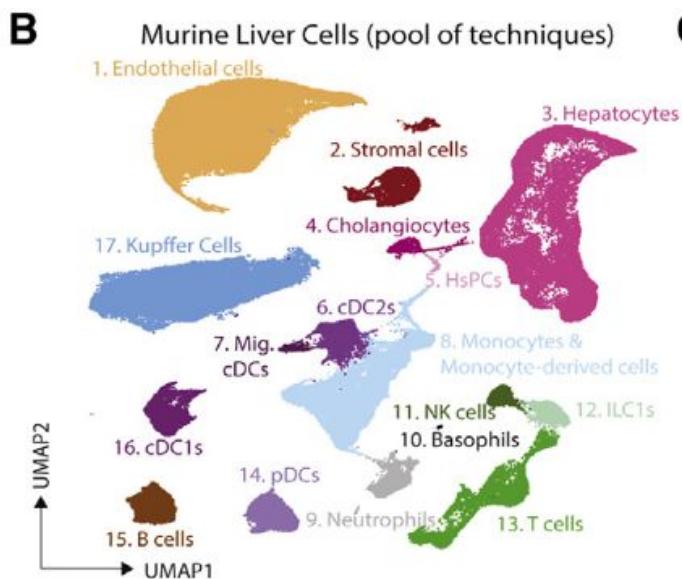
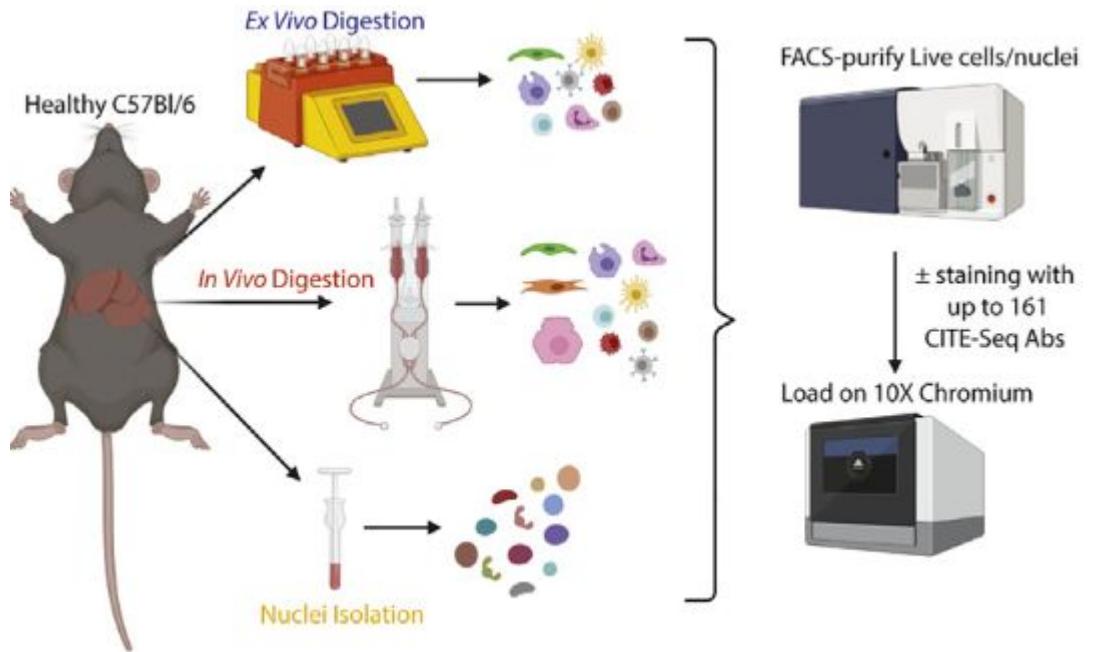
Capture Area with ~5000 Barcoded Spots



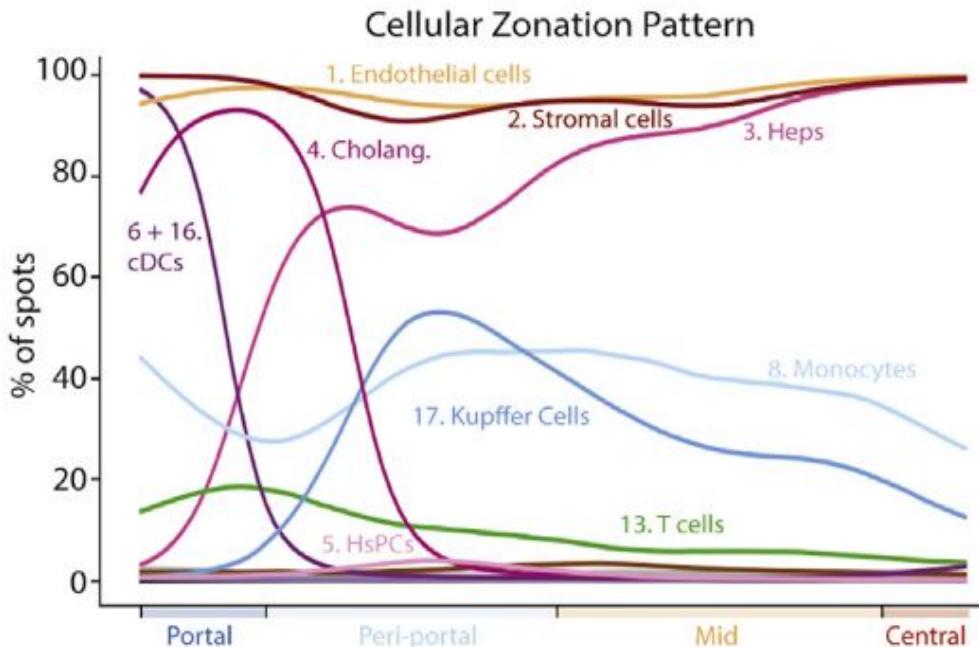
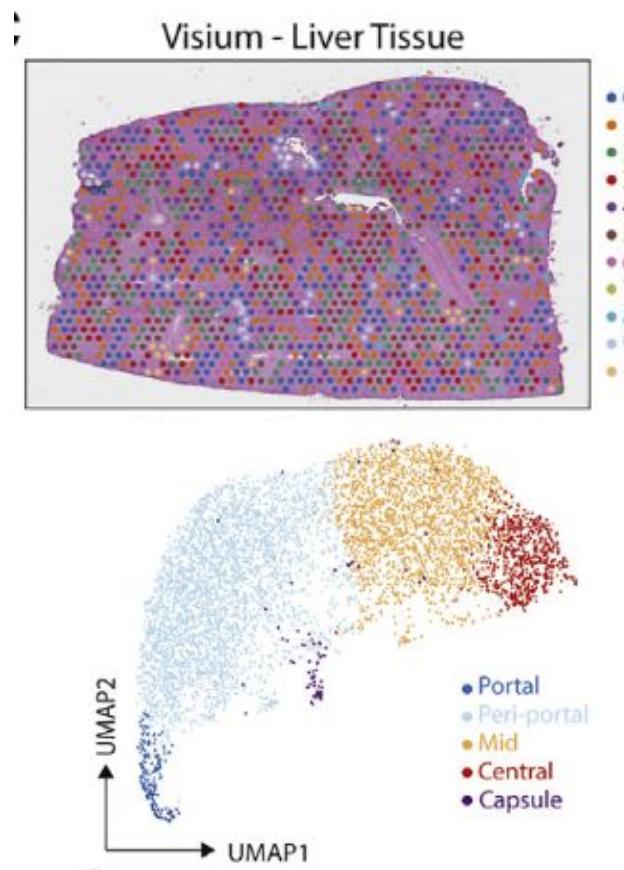
Visium Gene Expression Barcoded Spots



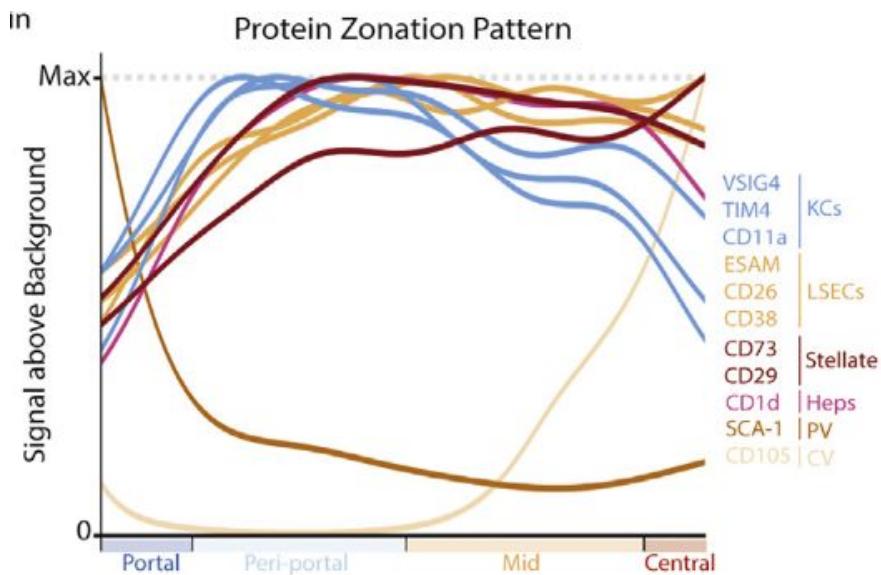
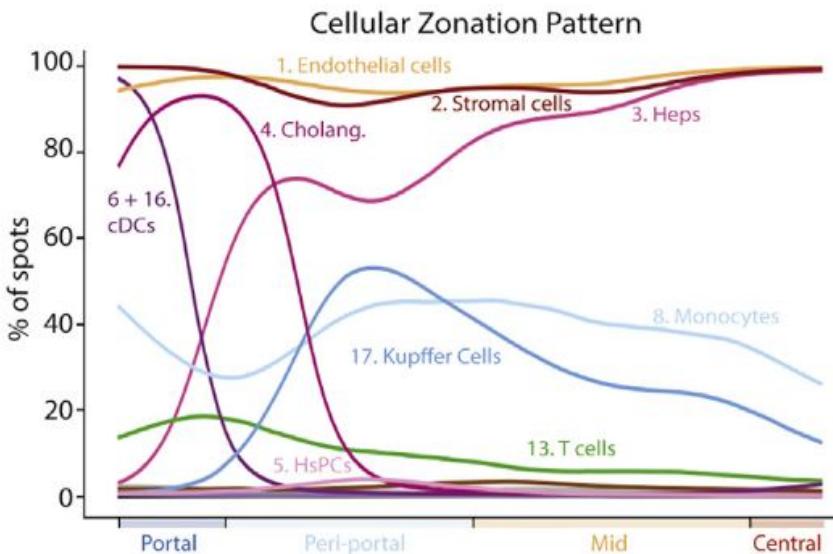
Spatial and single-cell expression of liver cells



Spatial and single-cell expression of liver cells



Spatial mRNA and protein expression data empowers digital pathology and biological understanding



Proteomics enables the elucidation of protein relations in the protein communities

